



OSPEDALE POLICLINICO SAN MARTINO  
UNIVERSITA' DEGLI STUDI DI GENOVA  
CLINICA EMATOLOGICA



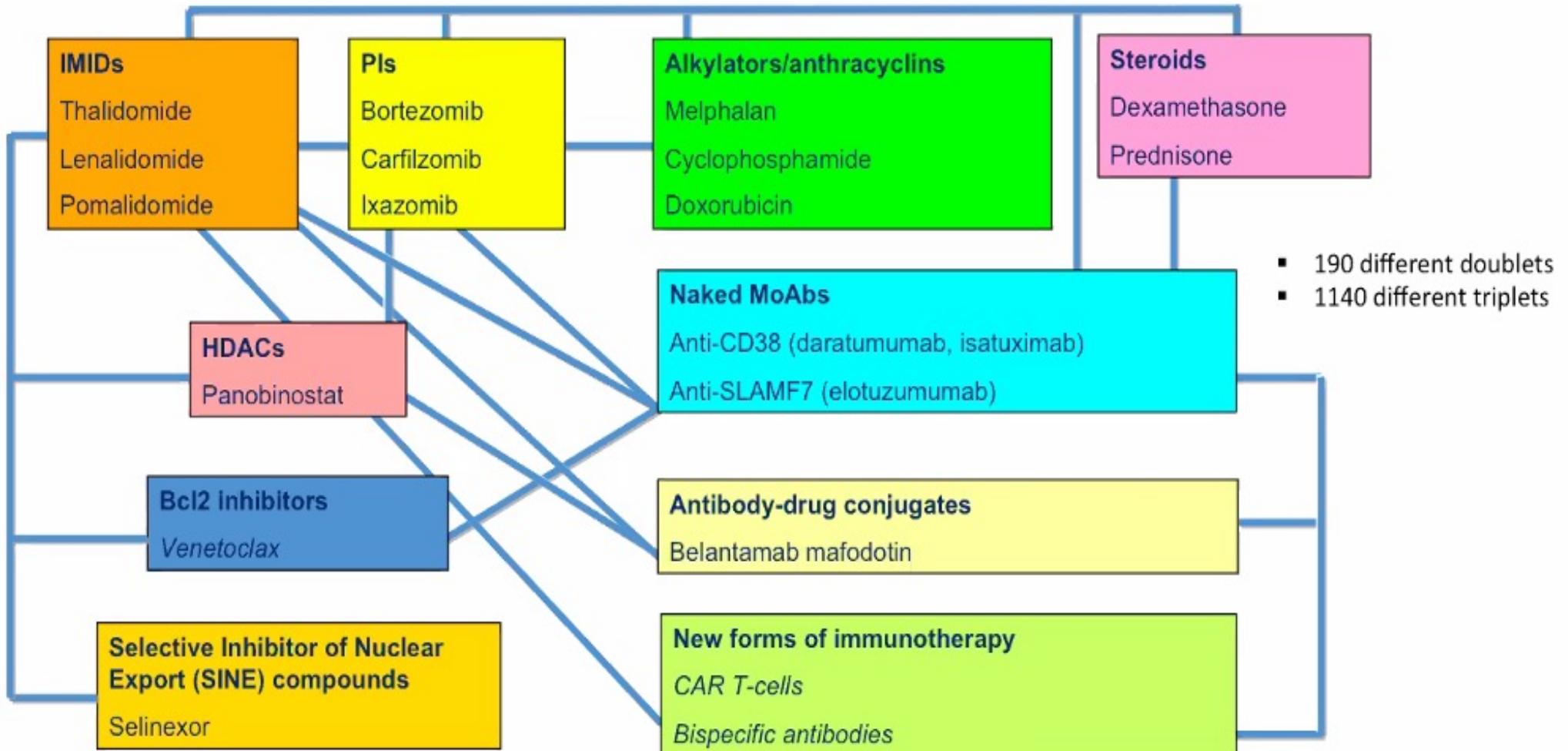
**Mercoledì 15 Dicembre 2021**

**GENOVA**  
Starhotels President

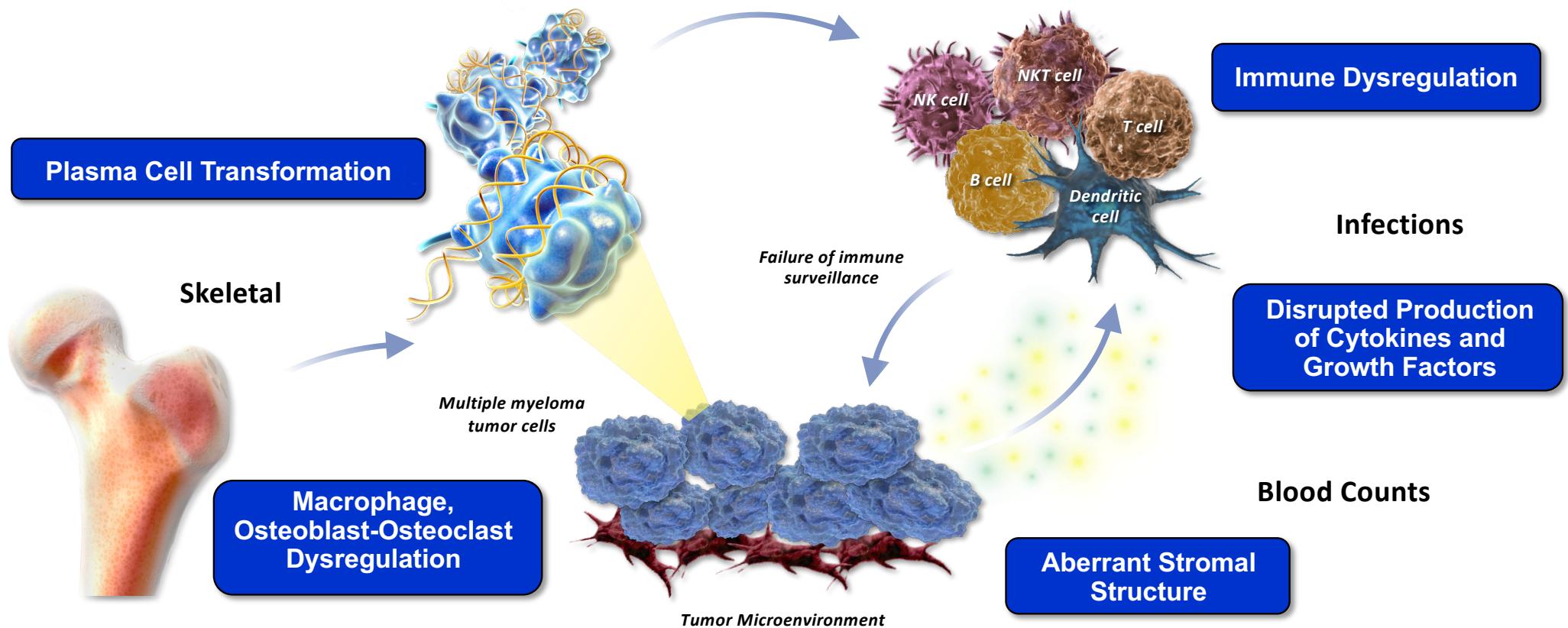
Michele Cea, MD

ANTICORPI MONOCLONALI ANTI-CD38 NEL MIELOMA MULTIPLO: MECCANISMO D'AZIONE ED UTILIZZO CLINICO

# Anti-MM agents: 2021



# Multiple Myeloma Pathogenesis



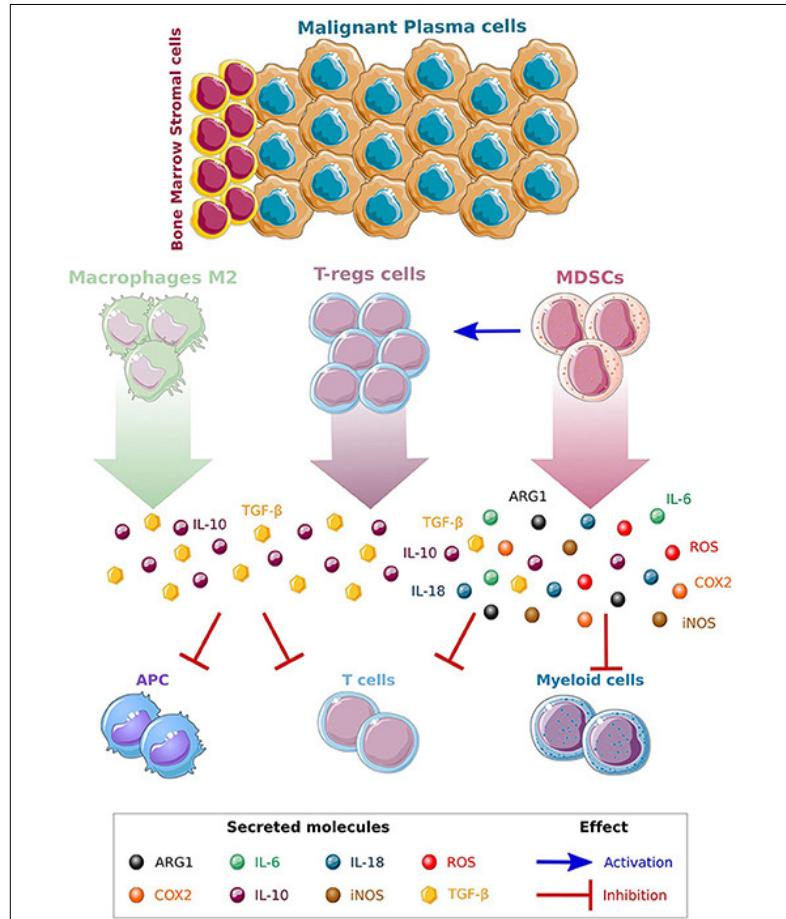
Cook G, Campbell JD. Blood Rev.1999

Kyle RA, et al. N Engl J Med. 2004

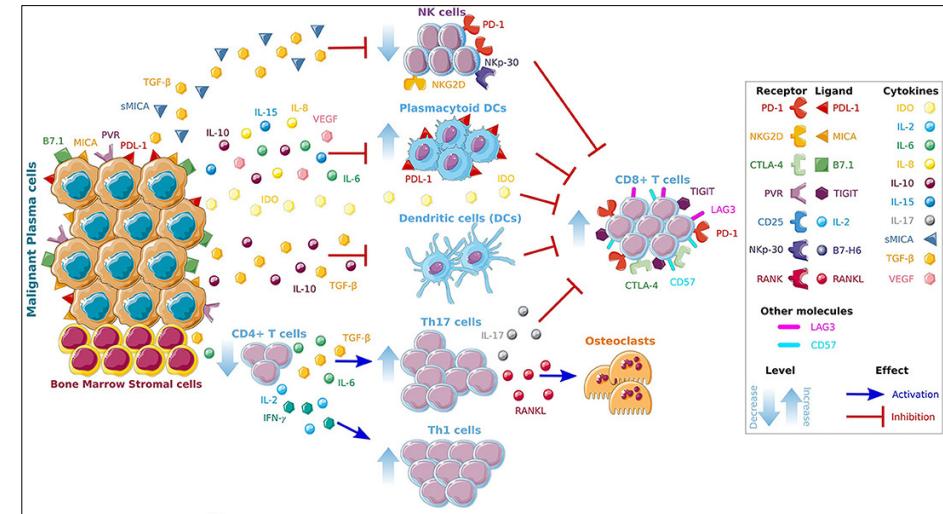
Raab MS, et al. Lancet. 2009

Morgan GJ, et al. Nat Rev Canc. 2012

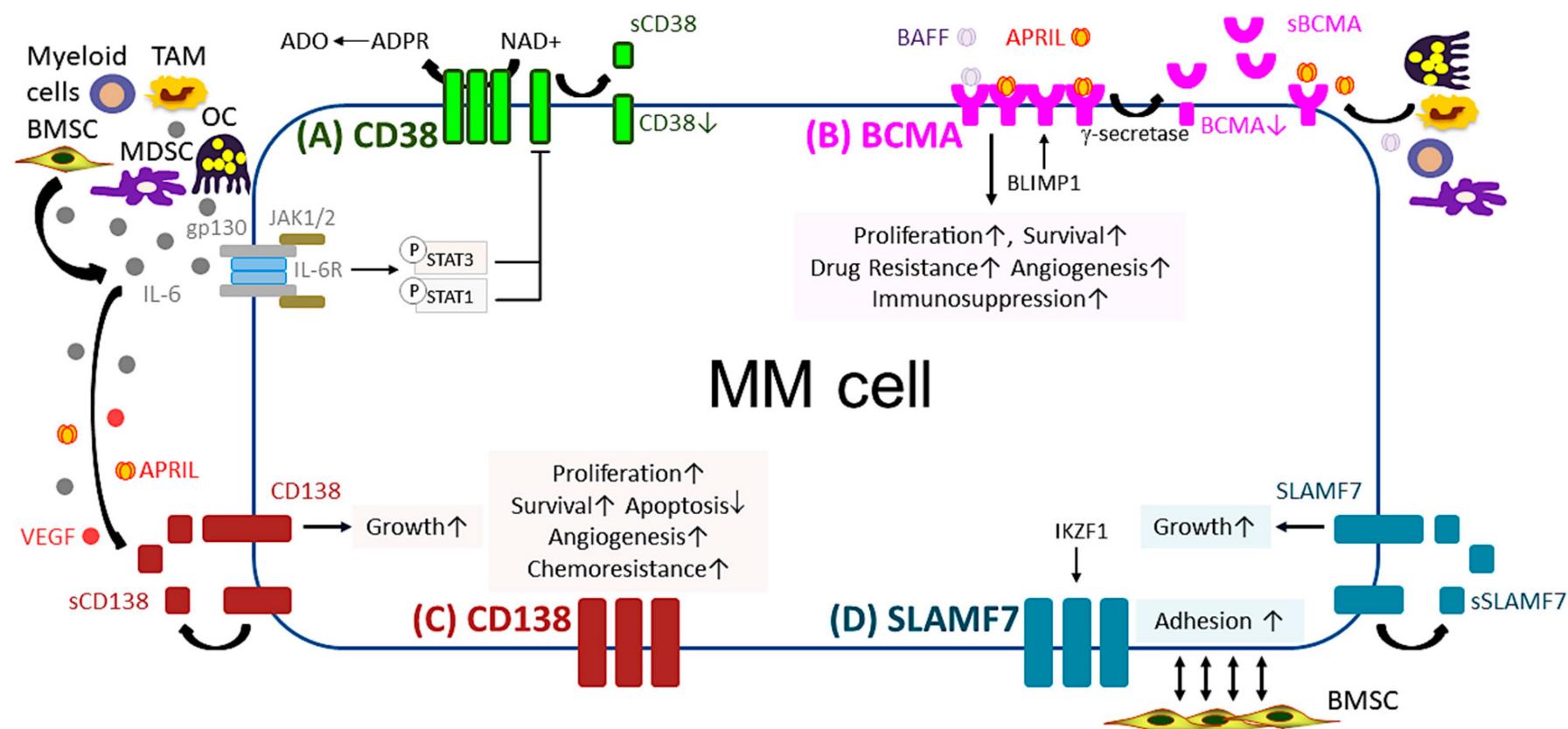
# Immune dysfunction in Multiple Myeloma



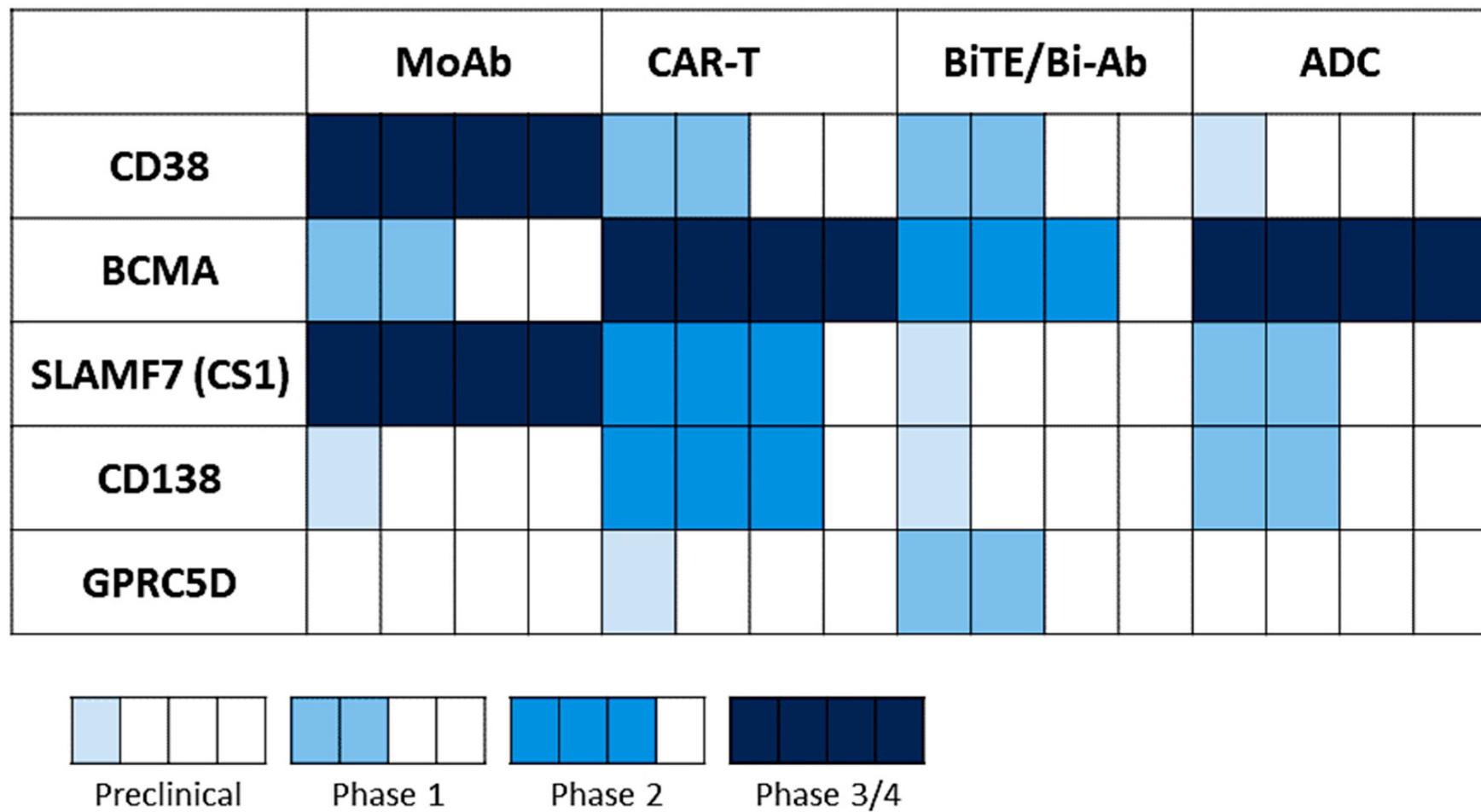
- **Contributes to disease progression**
- **Increases rate of infections**
- **Targets for therapy**



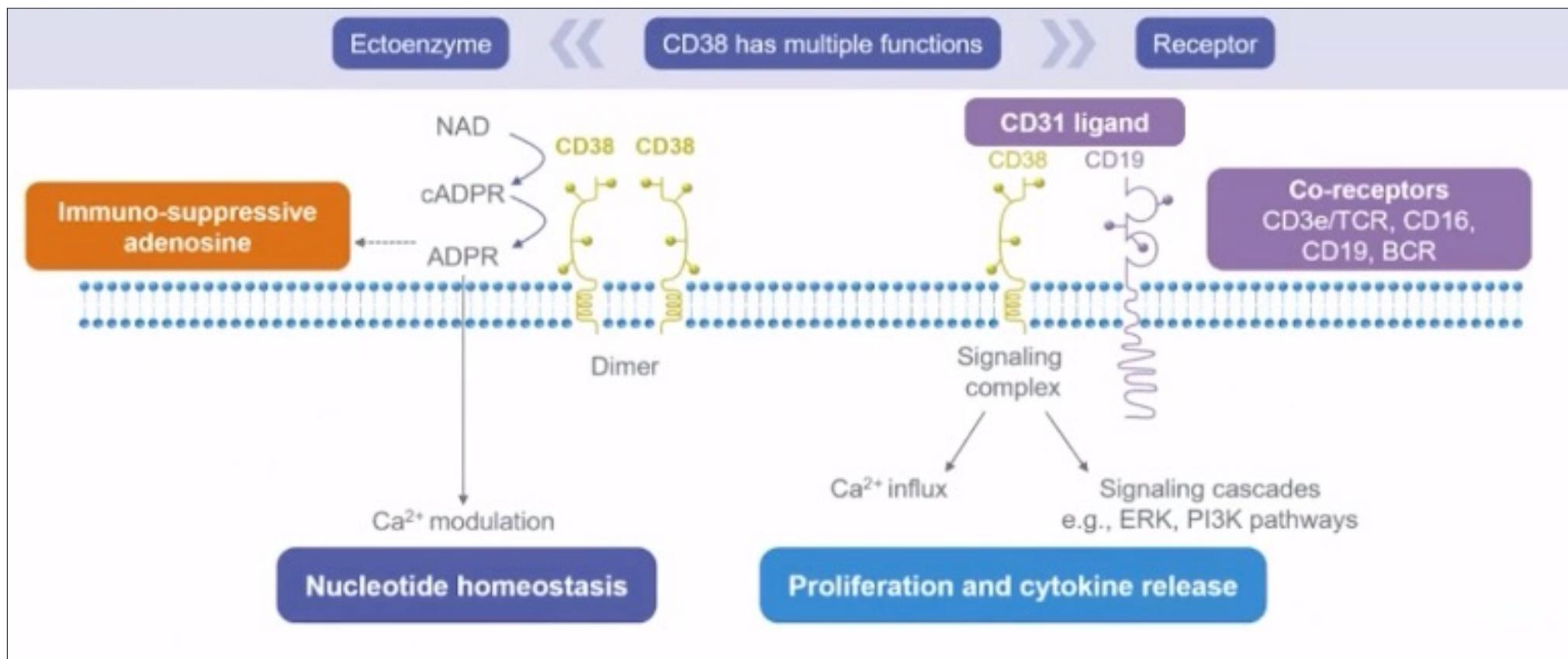
# Patho-biological function of MM target antigens commonly used in immunotherapies



## Statuses of various targeting immunotherapeutic agents under development in MM



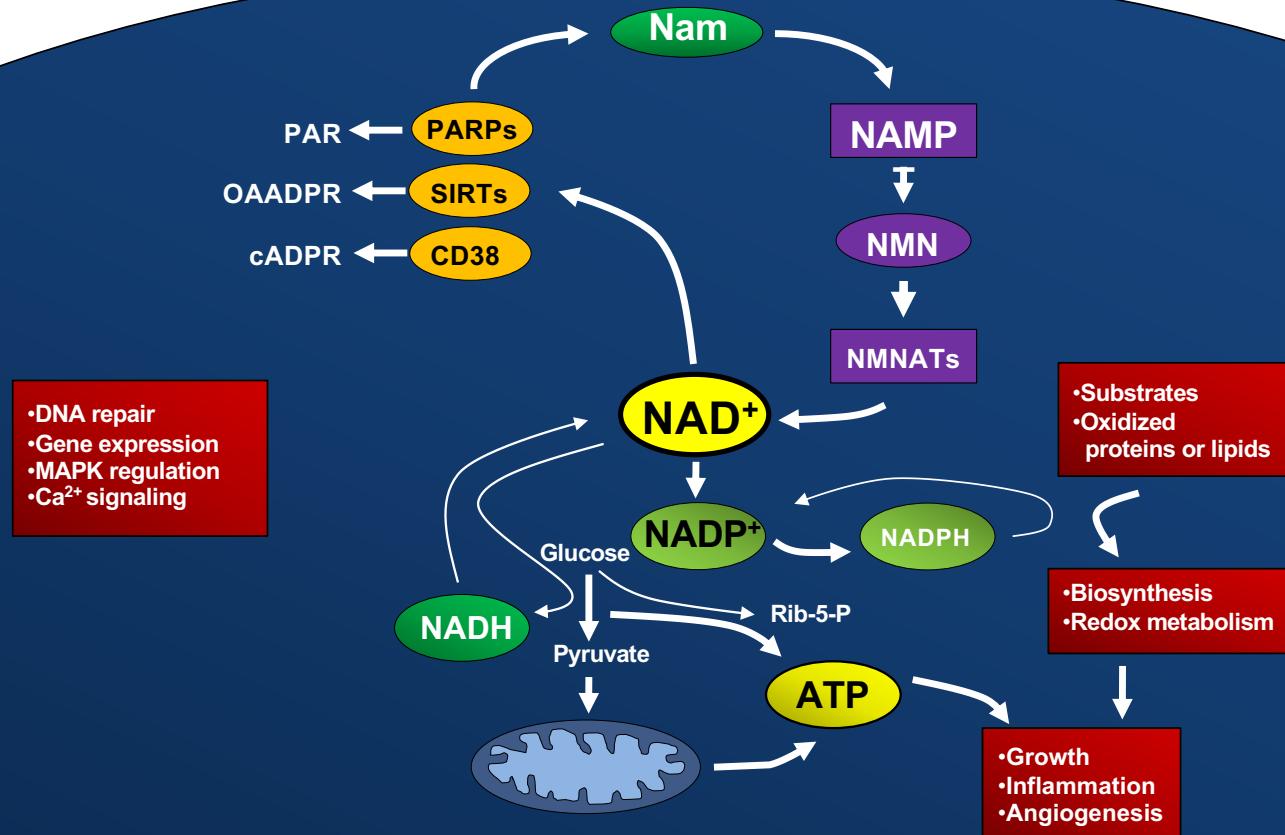
# CD38 is a transmembrane glycoprotein expressed by most Hemopoietic cells



cADPR, (cyclic) adenosine diphosphoribose; ERK, extracellular signal-regulated kinase;  
NAD nicotinamide adenine dinucleotide; PI3K, phosphatidylinositol 3 kinase

Adapted from Malavasi F, et al. *Physiol Rev*. 2008 Jul; 88(3):841-86  
Adapted from Chillemi A, et al. *Mol. Med.* 2013 May 20;19(1):99-108  
Adapted from Moreno-Garcia ME, et al. *J. Immunol.* 2005 Mar 1;174(5):2687-95

# Biological role of Nicotinamide Adenine Dinucleotide (NAD) in MM



Cea M. et al. Blood. 2010

Cea M. et al. Blood. 2012

Cagnetta A. et al. Blood. 2013

Cagnetta A. et al. Clin. Cancer Res. 2015

Becherini P. et al. abstract SIES 2021

Cea M. et al. Clin. Cancer Res. 2016

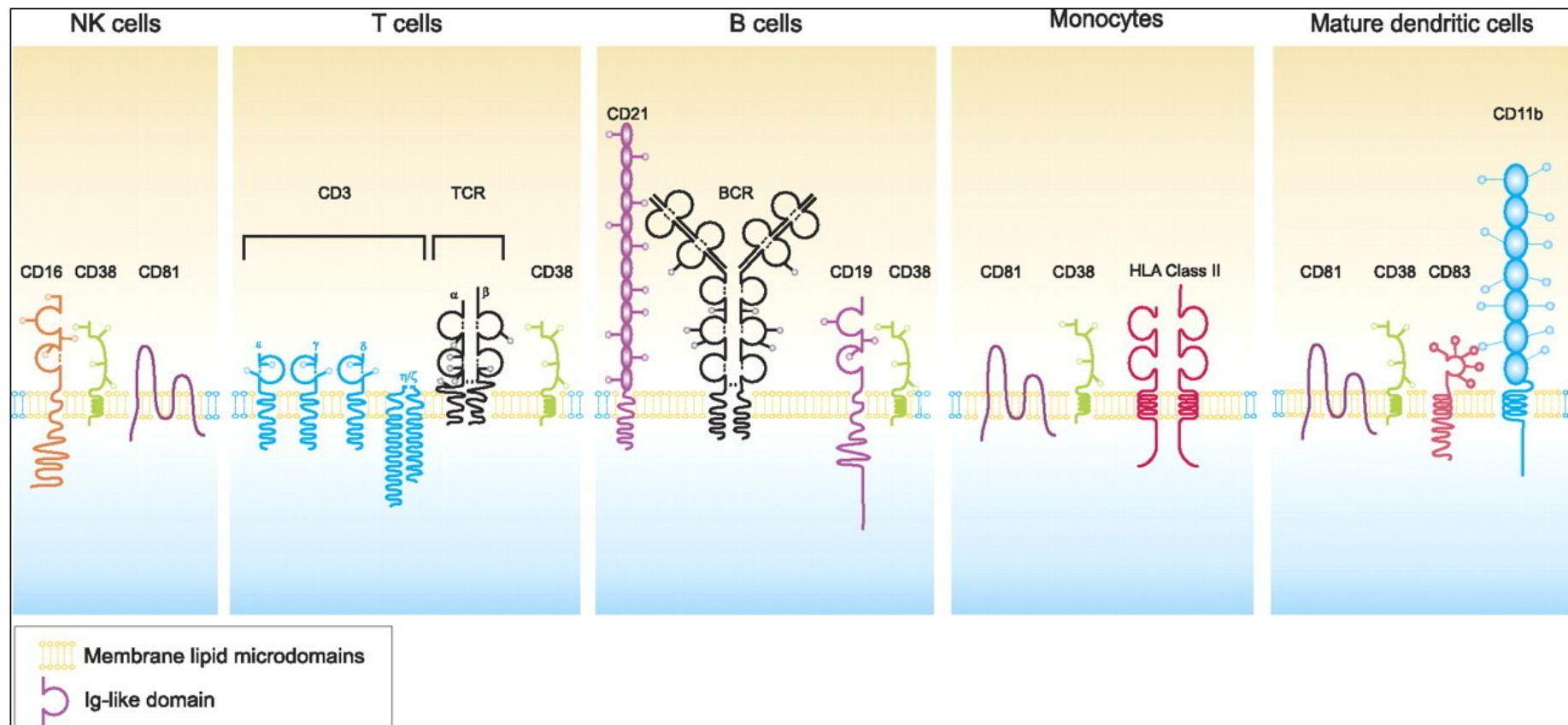
Cea M. et al. Blood. 2016

Cagnetta A. et al. Haematologica. 2018

Becherini P. et al. Cell. Metab. et al. 2021

Soncini D. et al. manuscript in preparation

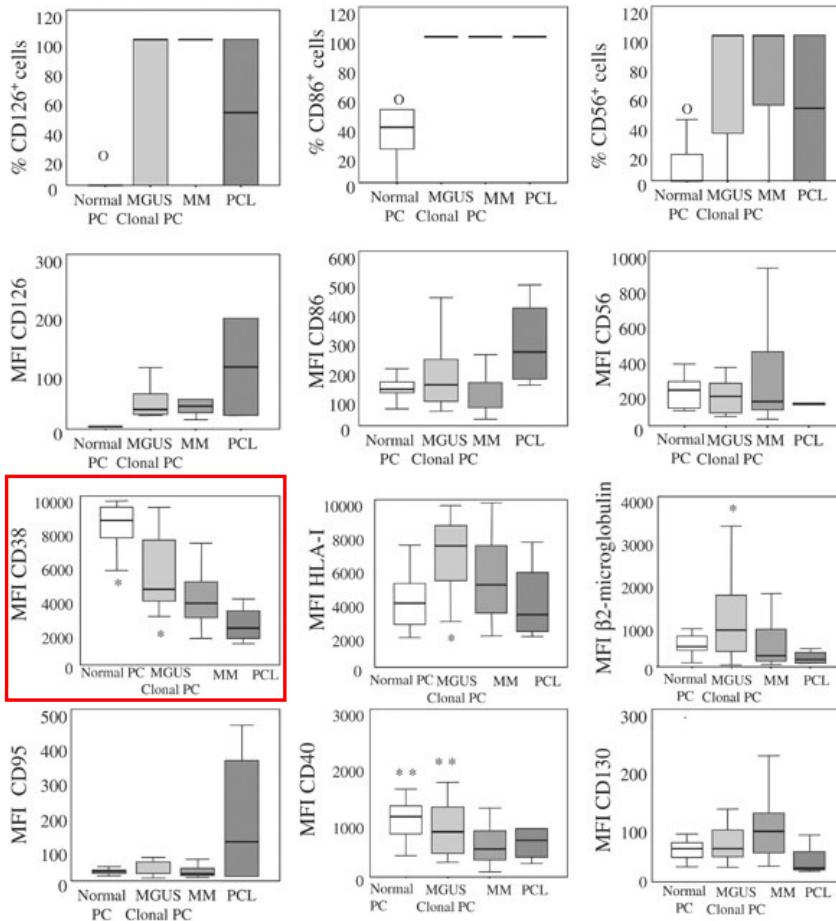
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# CD38 is variably expressed on MM cells



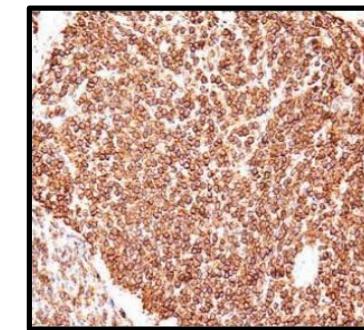
Monoclonal gammopathy of undetermined significance (MGUS)

Multiple Myeloma (MM)

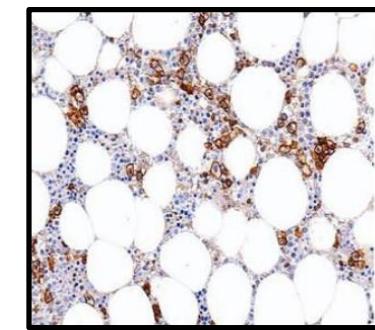
Plasma cells (PC)

Plasma cell leukemia (PCL)

In high density MM cells

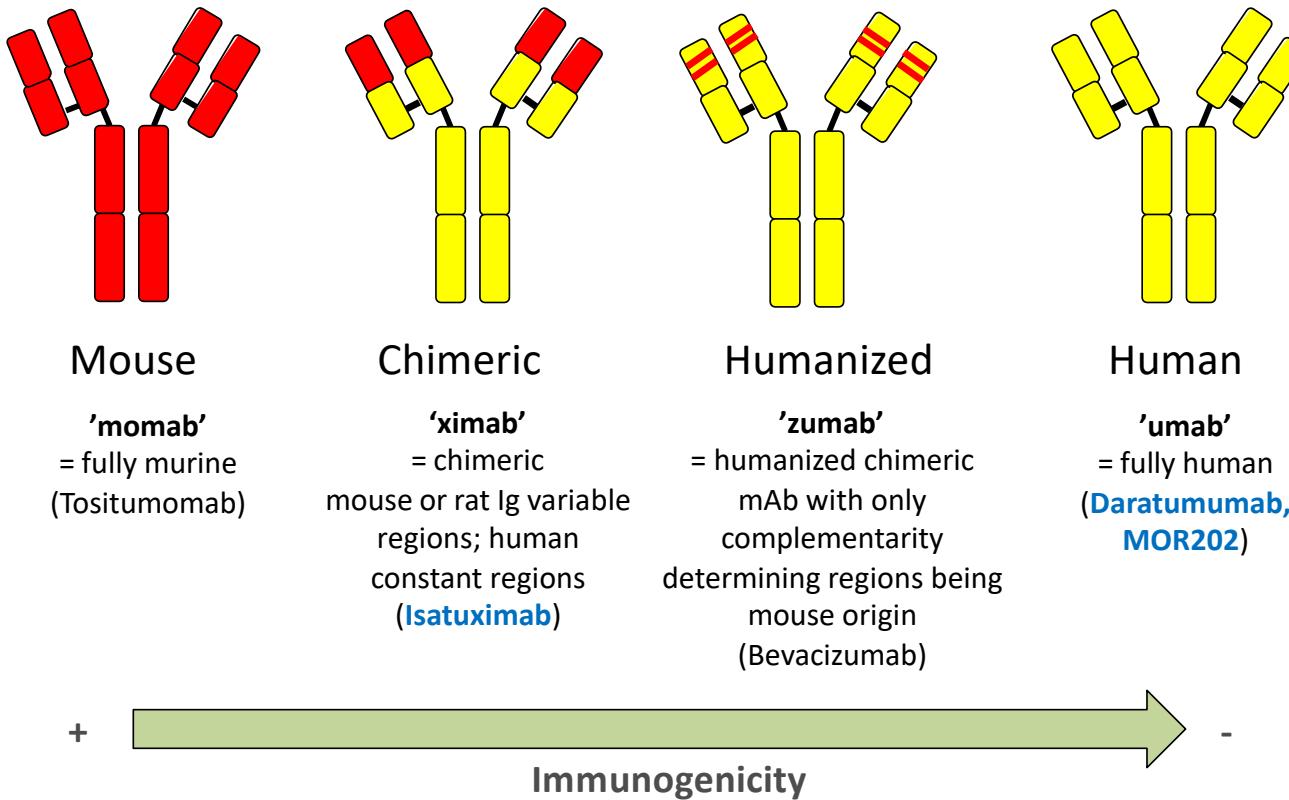


In low density MM cells



.....but also on other hematopoietic cells,  
and various non hematopoietic tissues

# Humanization of antibodies to overcome immunogenicity



# CD38-targeting Monoclonal Antibodies

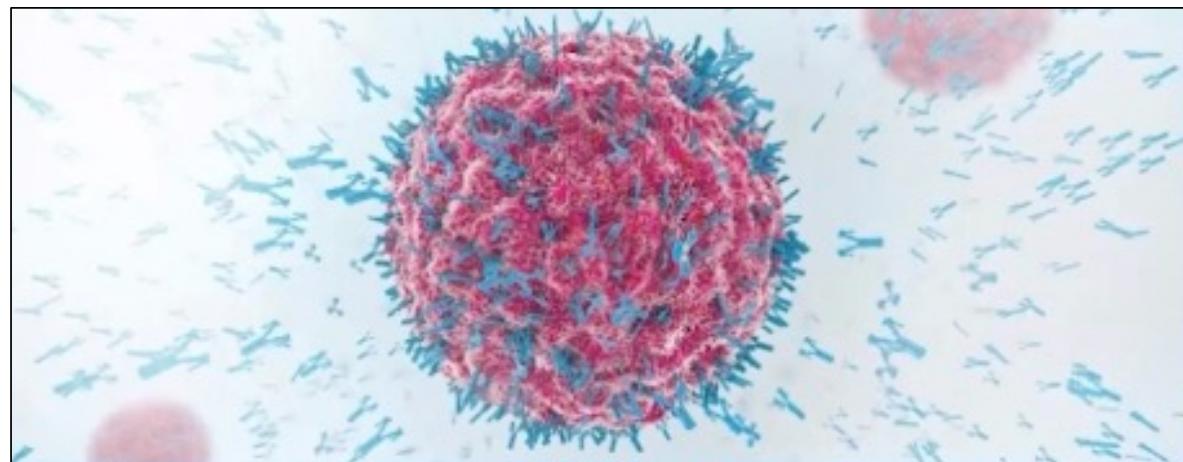
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## APPROVED

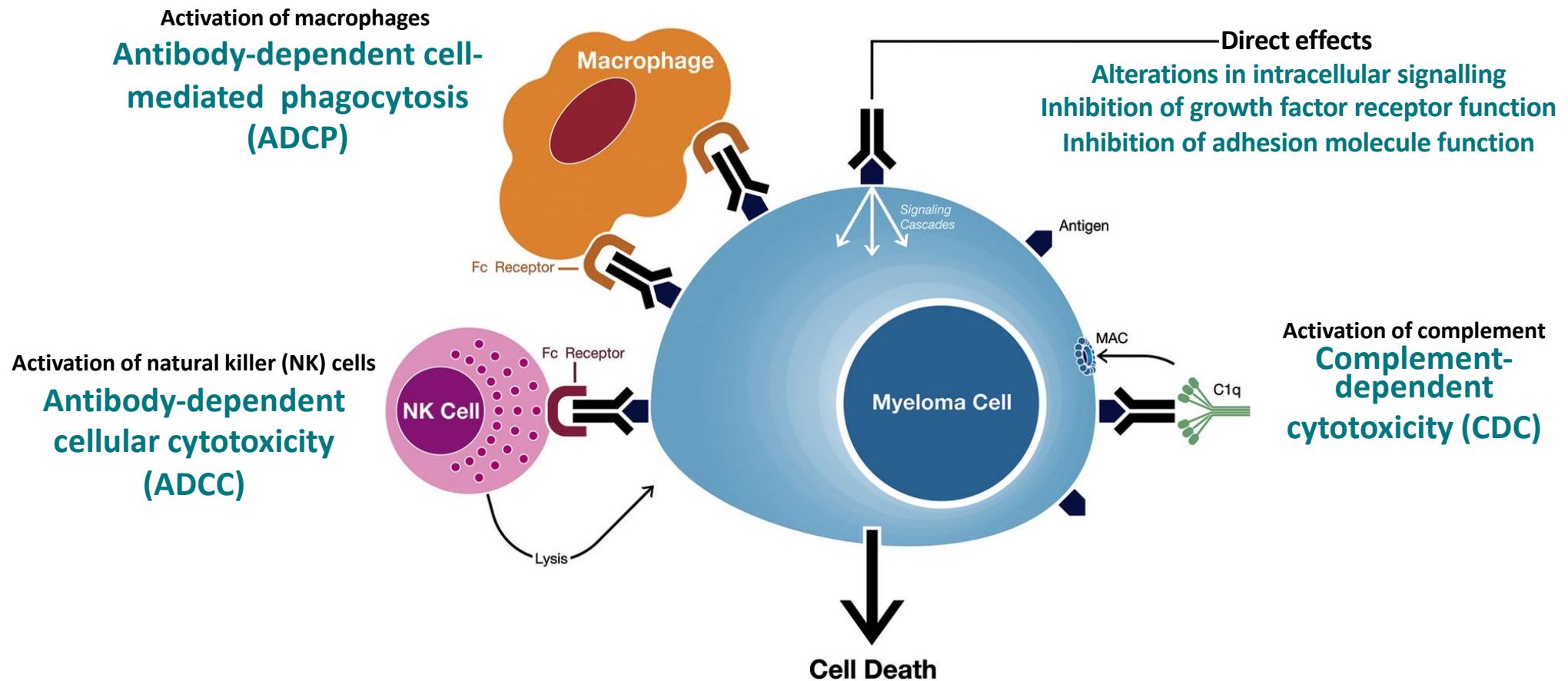
- DARATUMUMAB (DARA)
- ISATUXIMAB (SAR650984)

## NOT APPROVED

- MOR202 (MOR)
- TAK-079 (TAKEDA)



# Monoclonal Antibodies act through different MOA



# Summary of mechanisms of action of anti-CD38 mAbs

	DARA (human)	SAR (humanized*)	MOR (human)	TAK (humanized)
Binding	+++	+++	++	+++
CDC (max lysis)	+++	+	+	++
Phagocytosis	+++	+++	++	+++
ADCC (max lysis)	++	++	++	++
PCD direct	-	++	-	-
PDC crosslinking	+++	+++	+++	+++
Modulation ectoenzyme function	+	+++	-	+

Isatuximab

The clinical significance of these data is unknown. No interferences of clinical superiority should be made

\* n.d.: not determined

CDC: Complement-dependent cytotoxicity

ADCC: Antibody-dependent cell-mediated cytotoxicity

PCD: programmed cell death

Adapted from van de Donk NWCJ, et al. Blood 2018 Jan 4; 131(1): 13-29  
 Van de Donk NWCJ et al. Front. Immunol. 2018 Sep 20;9:2134

# in vitro comparison of Daratumumab with surrogate analogs of CD38 antibodies

	DARA	SAR	MOR	TAK
Binding	+++	+++	++	+++
CDC (max lysis)	+++	+	+	++
Phagocytosis	+++	+++	++	+++
ADCC (max lysis)	++	++	++	++
PCD direct	-	++	-	-
PDC crosslinking	+++	+++	+++	+++
Modulation ectoenzyme function	+	+++	-	+

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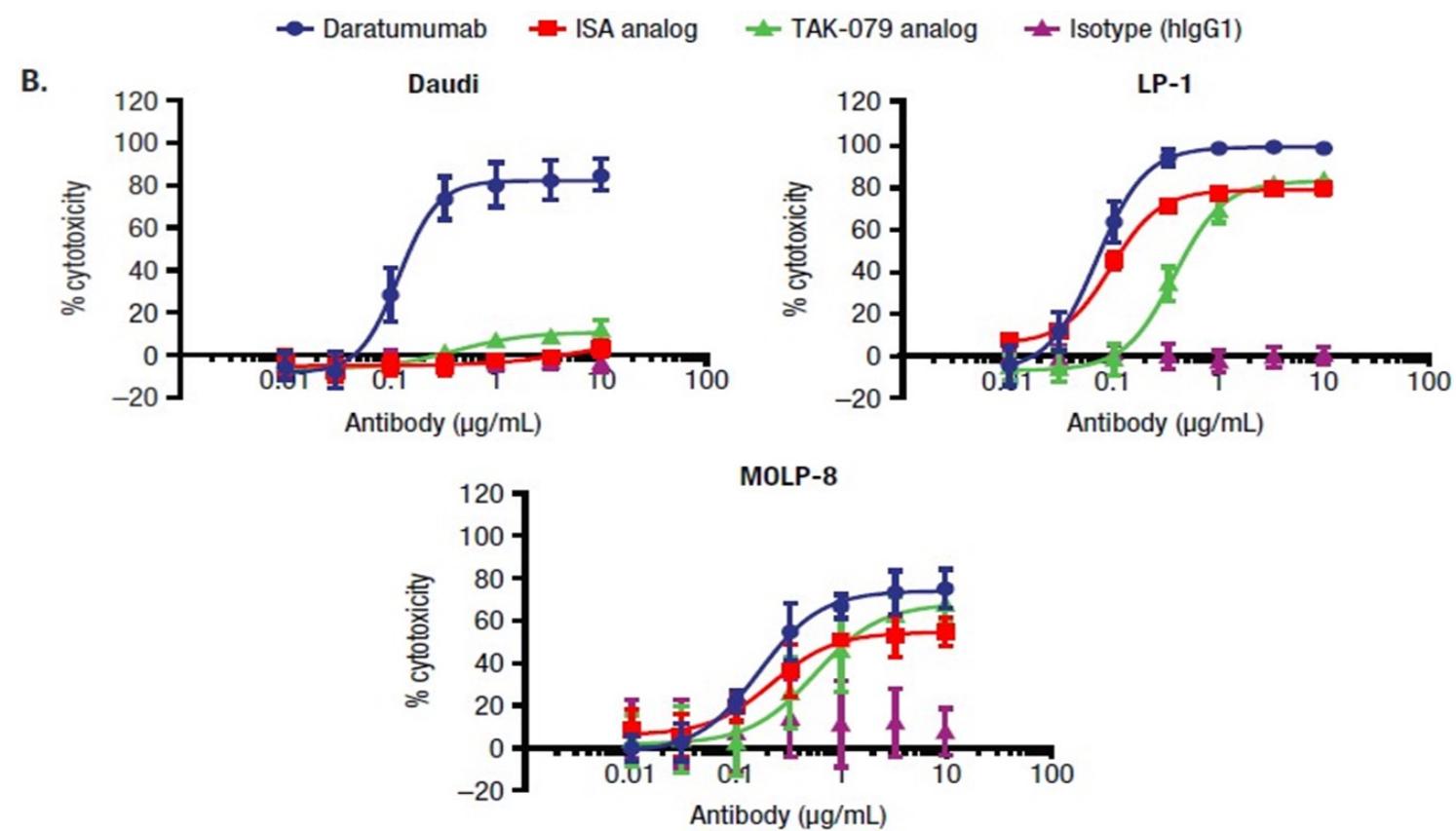
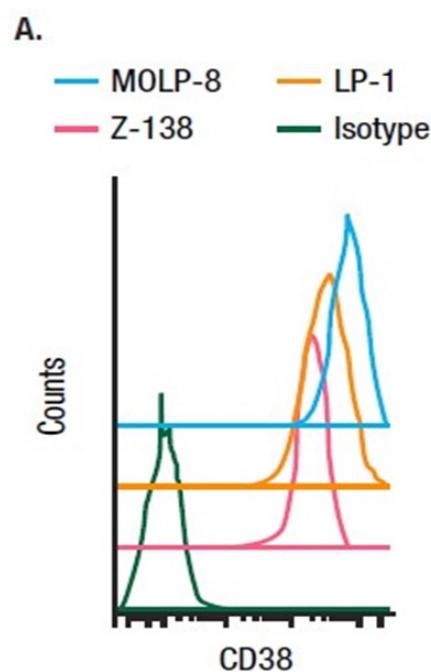
CDC: Complement-dependent cytotoxicity

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PCD: programmed cell death

Adapted from van de Donk NWCJ, et al. Blood 2018 Jan 4; 131(1): 13-29  
 Van de Donk NWCJ et al. Front. Immunol. 2018 Sep 20; 9:2134

# CDC Activity of Dara, Isa and TAK-079 in MM cell lines



# in vitro comparison of Daratumumab with surrogate analogs of CD38 antibodies

	DARA	SAR	MOR	TAK
Binding	+++	+++	++	+++
CDC (max lysis)	+++	+	+	++
Phagocytosis	+++	+++	++	+++
ADCC (max lysis)	++	++	++	++
PCD direct	-	++	-	-
PDC crosslinking	+++	+++	+++	+++
Modulation ectoenzyme function	+	+++	-	+

Isatuximab

\* n.d.: not determined

The clinical significance of these data is unknown. No interferences of clinical superiority should be made

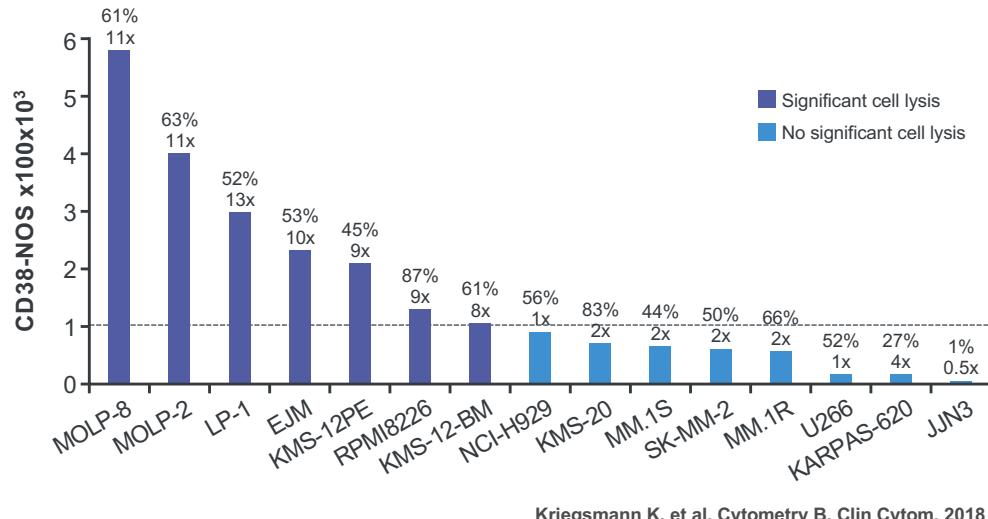
CDC: Complement-dependent cytotoxicity

ADCC: Antibody-dependent cell-mediated cytotoxicity

PCD: programmed cell death

Adapted from van de Donk NWCJ, et al. Blood 2018 Jan 4; 131(1): 13-29  
 Van de Donk NWCJ et al. Front. Immunol. 2018 Sep 20;:2134

# Induction of Antibody-Dependent Cell-mediated Cytotoxicity (ADCC) in MM cell lines



Daratumab induces ADCC in CD38-expressing MM cell lines	
Cell line	Daratumumab (% Lysis)
Daudi	61.5
MM1R	43.2
MM1S	47.5
28BM	23.8
28PE	6.1
12BM	15.4
12PE	39.1
RPMI 8226	54.2
LR5	39.2
DOX40	39.3
L363	52.1
H929	65.2
INA6	18.0
LP-1	54.6
UM-9	27.2
U266	0

Isatuximab induces ADCC against various cell lines as targets but requires a certain level of CD38 expression on the targets (left)

Daratumumab also shows ADCC activity against various cell lines with higher levels of CD38 (right)

- Both isatuximab and daratumumab induce ADCC against CD38 expressing cells
- Anti-CD38 antibodies-induced ADCC requires a certain level of CD38 expression on target cells
- Due to variation of data resources (e.g. different experimental systems), difference in ADCC induction between isatuximab and daratumumab still needs further cellular and molecular characterization

de Weers M, et al. J Immunol. 2011

# in vitro comparison of Daratumumab with surrogate analogs of CD38 antibodies

	DARA	SAR	MOR	TAK
Binding	+++	+++	++	+++
CDC (max lysis)	+++	+	+	++
Phagocytosis	+++	+++	++	+++
ADCC (max lysis)	++	++	++	++
PCD direct	-	++	-	-
PDC crosslinking	+++	+++	+++	+++
Modulation ectoenzyme function	+	+++	-	+

Isatuximab

The clinical significance of these data is unknown. No interferences of clinical superiority should be made

\* n.d.: not determined

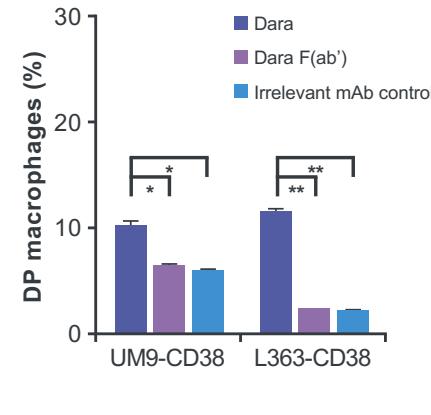
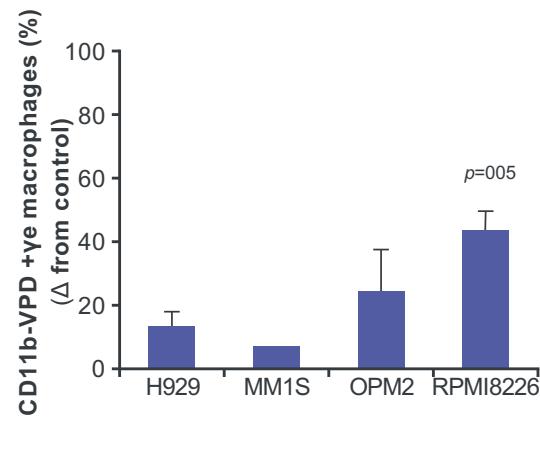
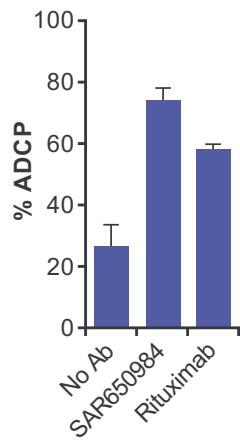
CDC: Complement-dependent cytotoxicity

ADCC: Antibody-dependent cell-mediated cytotoxicity

PCD: programmed cell death

Adapted from van de Donk NWCJ, et al. Blood 2018 Jan 4; 131(1): 13-29  
 Van de Donk NWCJ et al. Front. Immunol. 2018 Sep 20; 9:2134

# Induction of Antibody-Dependent Cellular Phagocytosis (ADCP) in MM cell lines



Isatuximab is shown to induce potent ADCP activity at the equivalent level induced by rituximab (left).

Isatuximab-induced ADCP is more prominent against cells with higher CD38 expression (center).

Daratumumab can induce ADCP against cells overexpressed CD38 (right)

- Both isatuximab and daratumumab induce ADCP against CD38 expressing cells
- Anti-CD38 antibodies-induced ADCP requires certain level of CD38 expression on target cells
- Due to variation of data resources (e.g. different experimental systems), difference in ADCP induction between isatuximab and daratumumab still needs further cellular and molecular characterization

\*p<0.001; \*\*p<0.0001. ADCP, antibody-dependent cellular phagocytosis; DP, double positive; mAb, monoclonal antibody; MM, multiple myeloma.

# in vitro comparison of Daratumumab with surrogate analogs of CD38 antibodies

	DARA	SAR	MOR	TAK
Binding	+++	+++	++	+++
CDC (max lysis)	+++	+	+	++
Phagocytosis	+++	+++	++	+++
ADCC (max lysis)	++	++	++	++
PCD direct	-	++	-	-
PDC crosslinking	+++	+++	+++	+++
Modulation ectoenzyme function	+	+++	-	+

Isatuximab

The clinical significance of these data is unknown. No interferences of clinical superiority should be made

\* n.d.: not determined

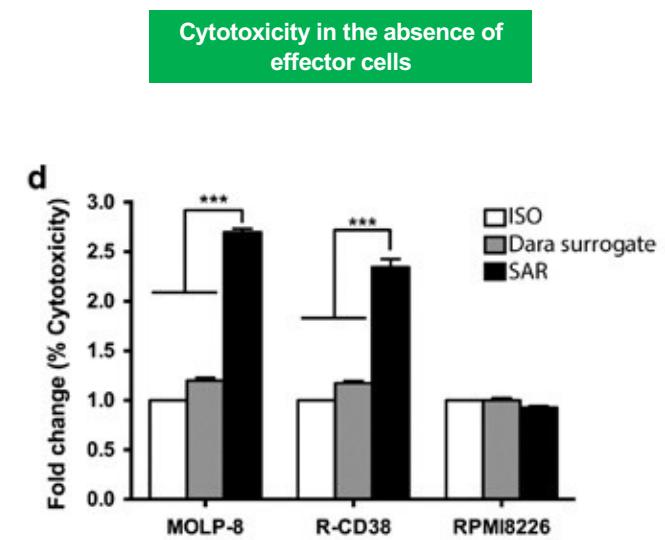
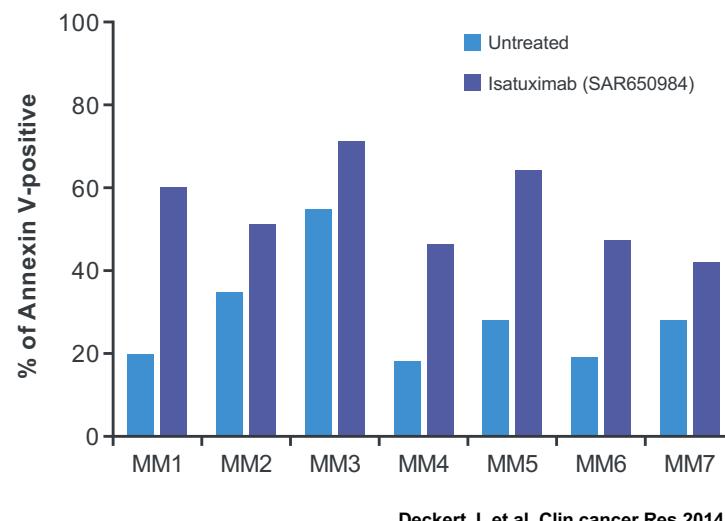
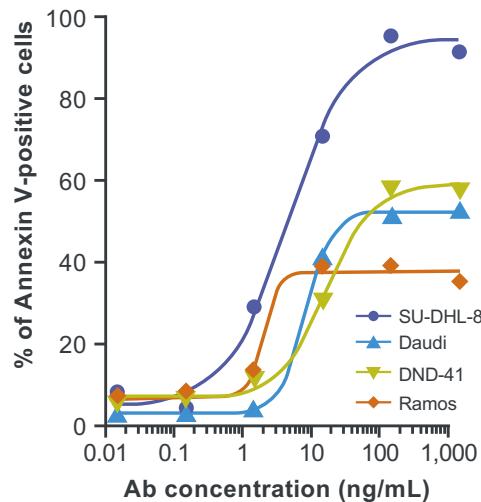
CDC: Complement-dependent cytotoxicity

ADCC: Antibody-dependent cell-mediated cytotoxicity

PCD: programmed cell death

Adapted from van de Donk NWCJ, et al. Blood 2018 Jan 4; 131(1): 13-29  
 Van de Donk NWCJ et al. Front. Immunol. 2018 Sep 20;:2134

## In vitro preclinical studies showed greater apoptosis (PCD) with isa compared with currently available CD38 mAbs



# in vitro comparison of Daratumumab with surrogate analogs of CD38 antibodies

	DARA	SAR	MOR	TAK
Binding	+++	+++	++	+++
CDC (max lysis)	+++	+	+	++
Phagocytosis	+++	+++	++	+++
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PCD direct	-	++	-	-
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Isatuximab

The clinical significance of these data is unknown. No interferences of clinical superiority should be made

\* n.d.: not determined

CDC: Complement-dependent cytotoxicity

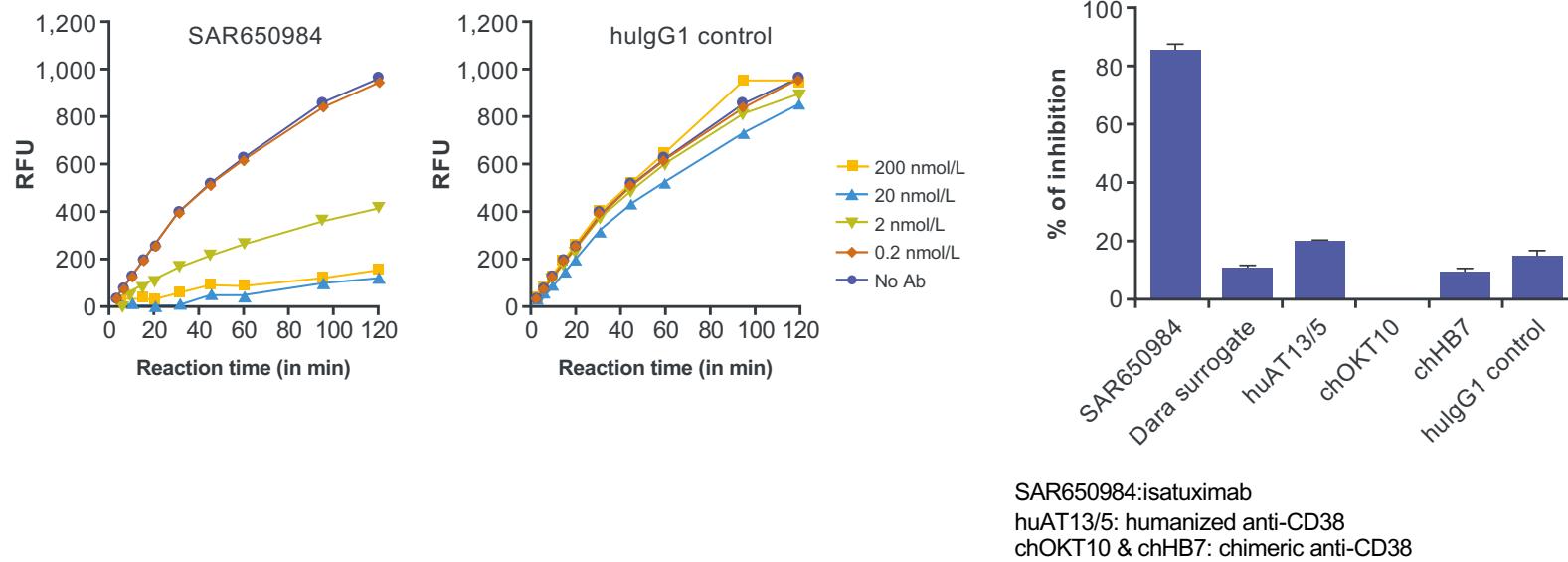
ADCC: Antibody-dependent cell-mediated cytotoxicity

PCD: programmed cell death

Adapted from van de Donk NWCJ, et al. Blood 2018 Jan 4; 131(1): 13-29  
 Van de Donk NWCJ et al. Front. Immunol. 2018 Sep 20;9::2134

# Isatuximab has demonstrated significant inhibition of CD38 ectoenzyme activity

Potent inhibition of the ADP ribosyl-cyclase enzymatic activity of human CD38 by isatuximab

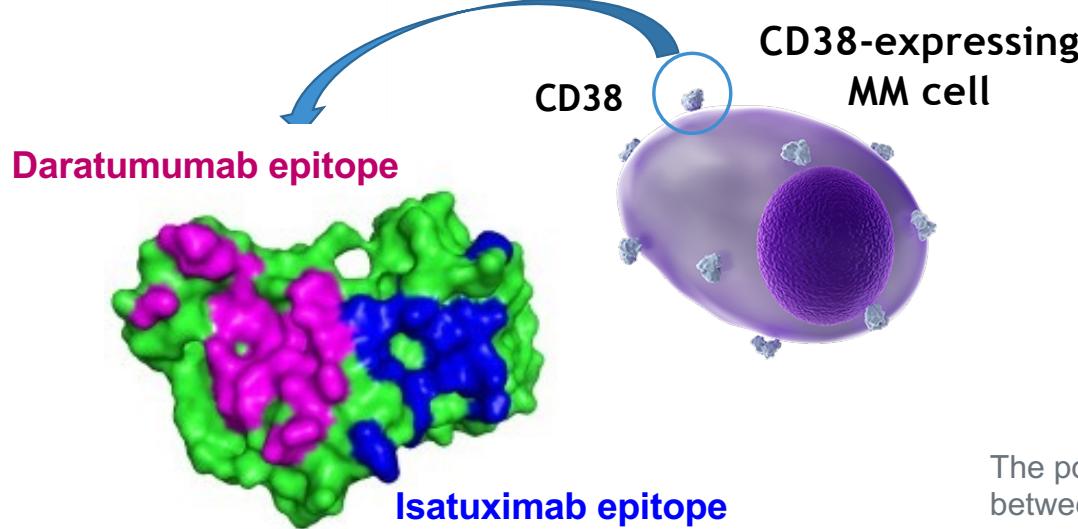


Isatuximab suppresses ADP ribosyl-cyclase activity of recombinant huCD38 in a dose-dependent manner (left) while the control isotype-matched hulgG1 does not (center).

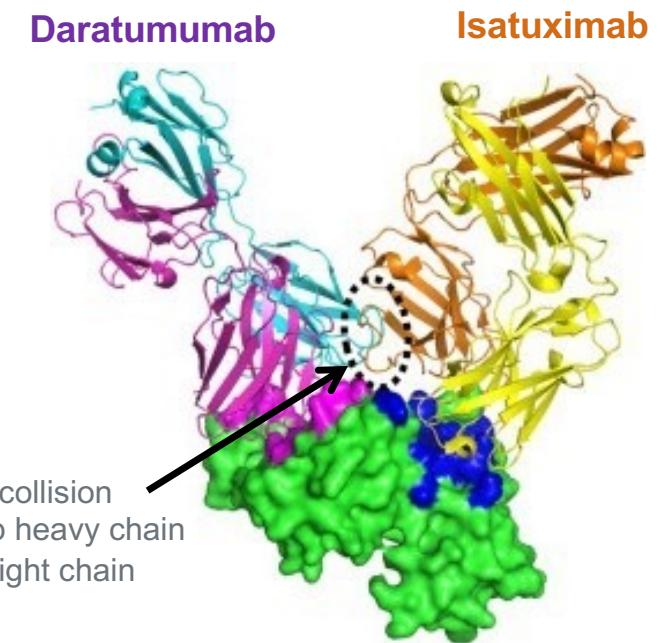
The degree of inhibition of CD38 cyclase activity by isatuximab is much superior to the other anti-huCD38 monoclonal antibodies including daratumumab surrogate (Ab005) (right).

- Isatuximab is shown to inhibit CD38 ADP-ribosyl cyclase activity in a dose-dependent manner
- The degree of CD38 ectoenzymatic activity inhibition by isatuximab is much superior to the other huCD38 targeting antibodies

## Molecular basis of interactions between anti-CD38 antibodies and huCD38:



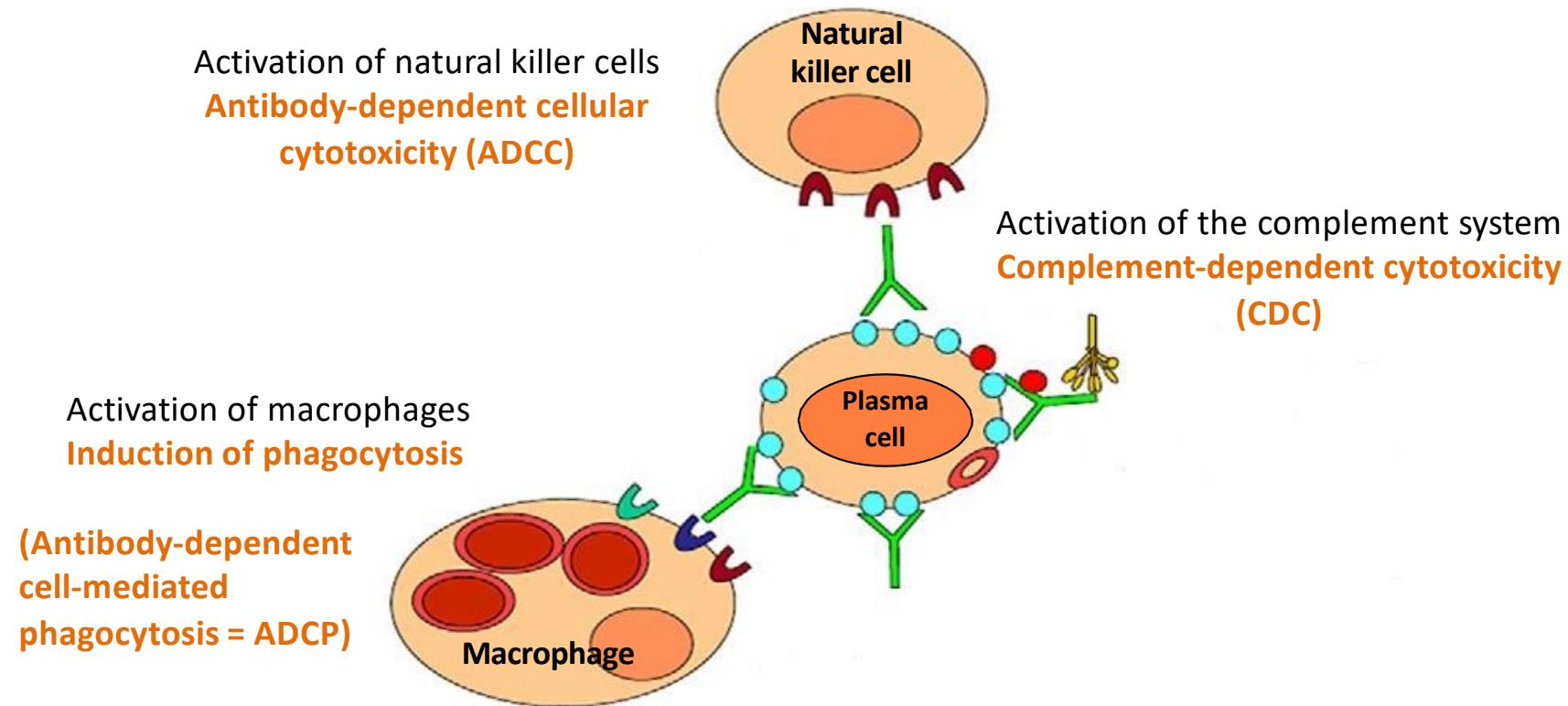
**The binding modes of isatuximab and daratumumab**



The possible steric collision  
between isatuximab heavy chain  
and daratumumab light chain

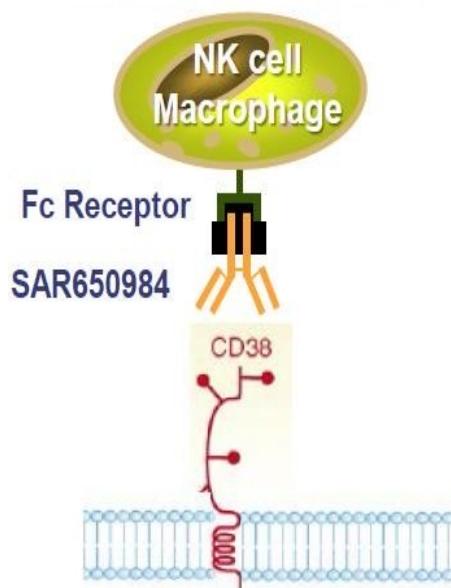
**The epitopes of huCD38 interacting with isatuximab and daratumumab paratopes are distinct**

# Daratumumab (DARA): MOA

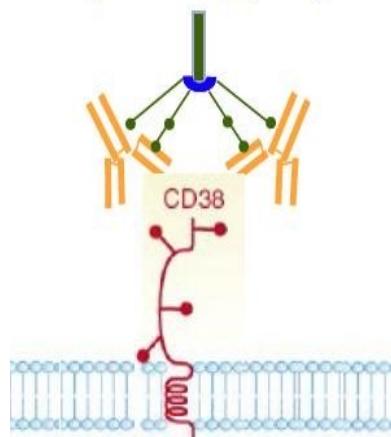


# Isatuximab (SAR650984): MOA

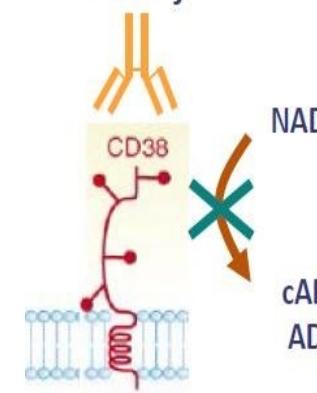
Antibody-dependent  
cellular cytotoxicity (ADCC)  
and phagocytosis (ADCP)



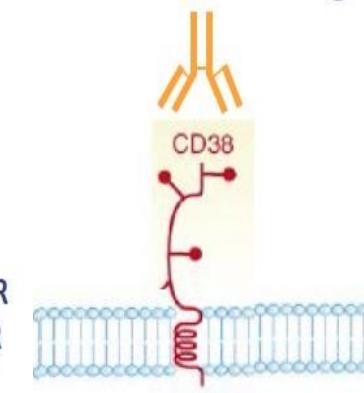
Complement-dependent  
cytotoxicity (CDC)



Inhibition of  
CD38 enzyme  
activity



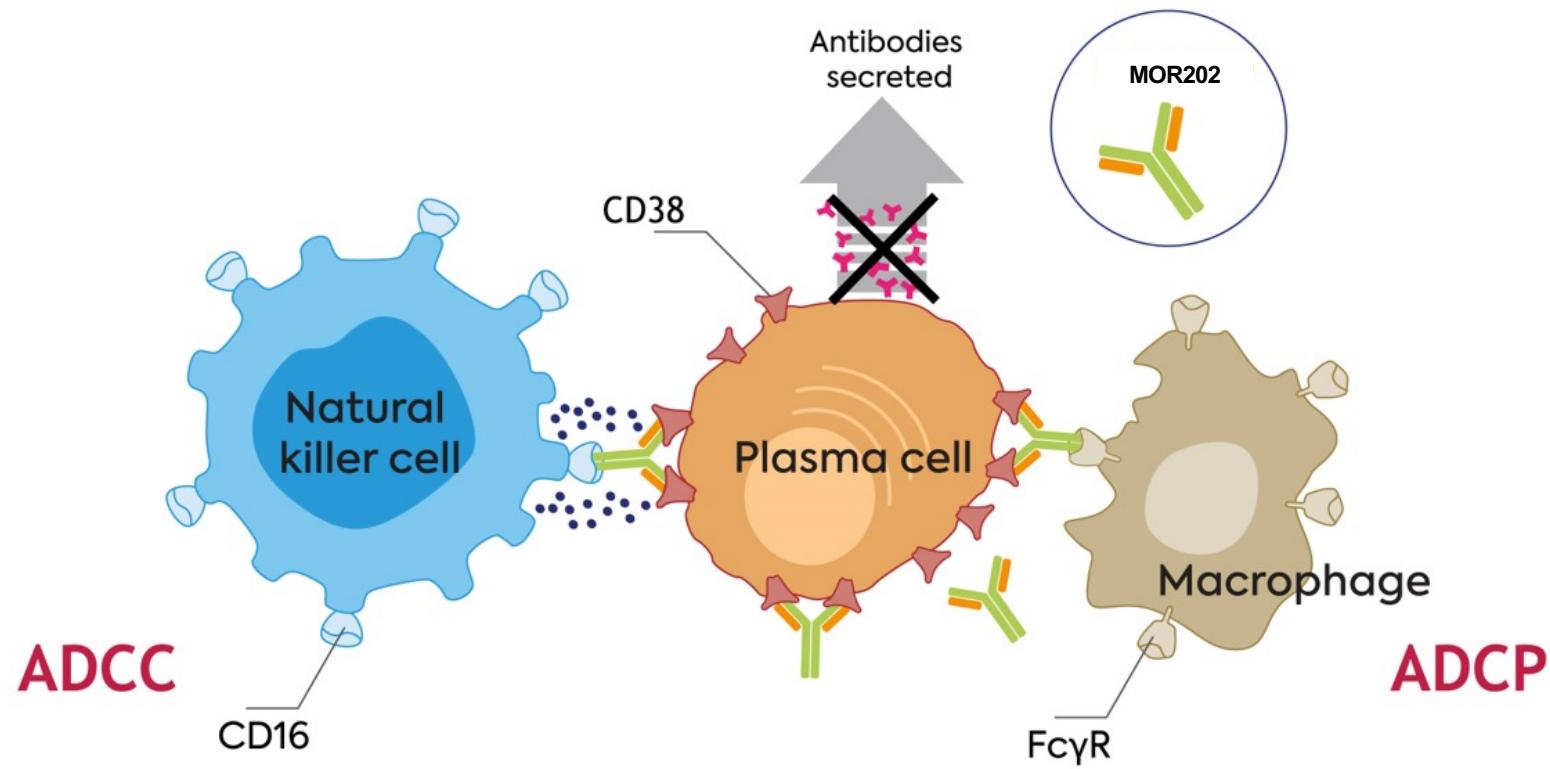
Direct apoptosis  
without crosslinking



Canonical and lysosome-dependent  
cell death

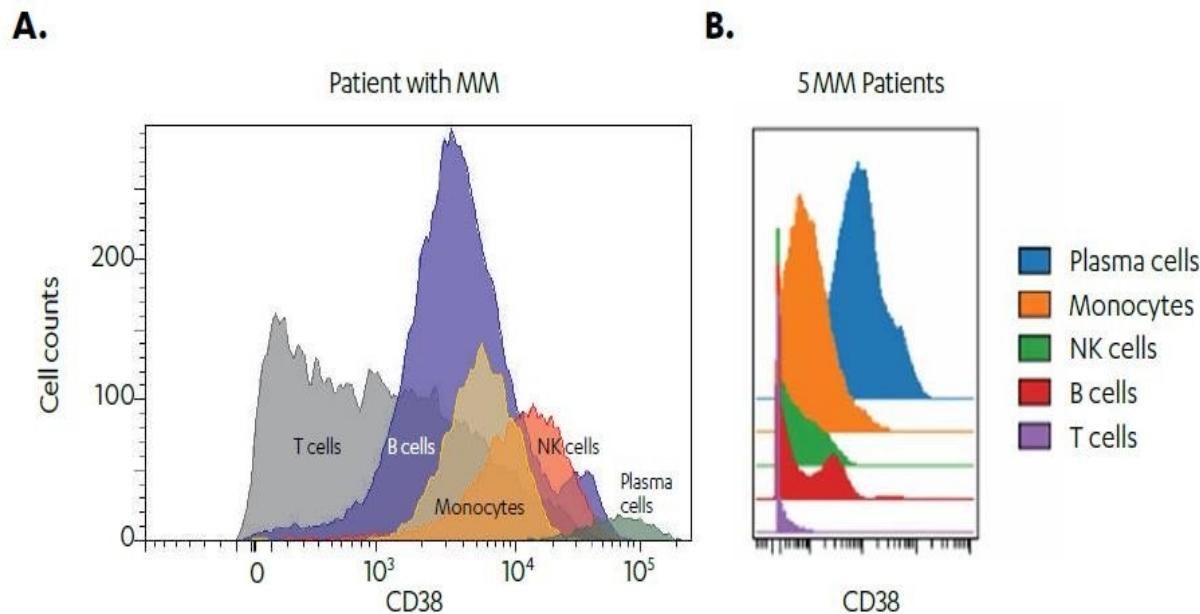
## MOR202 (CD38) mAb: MOA

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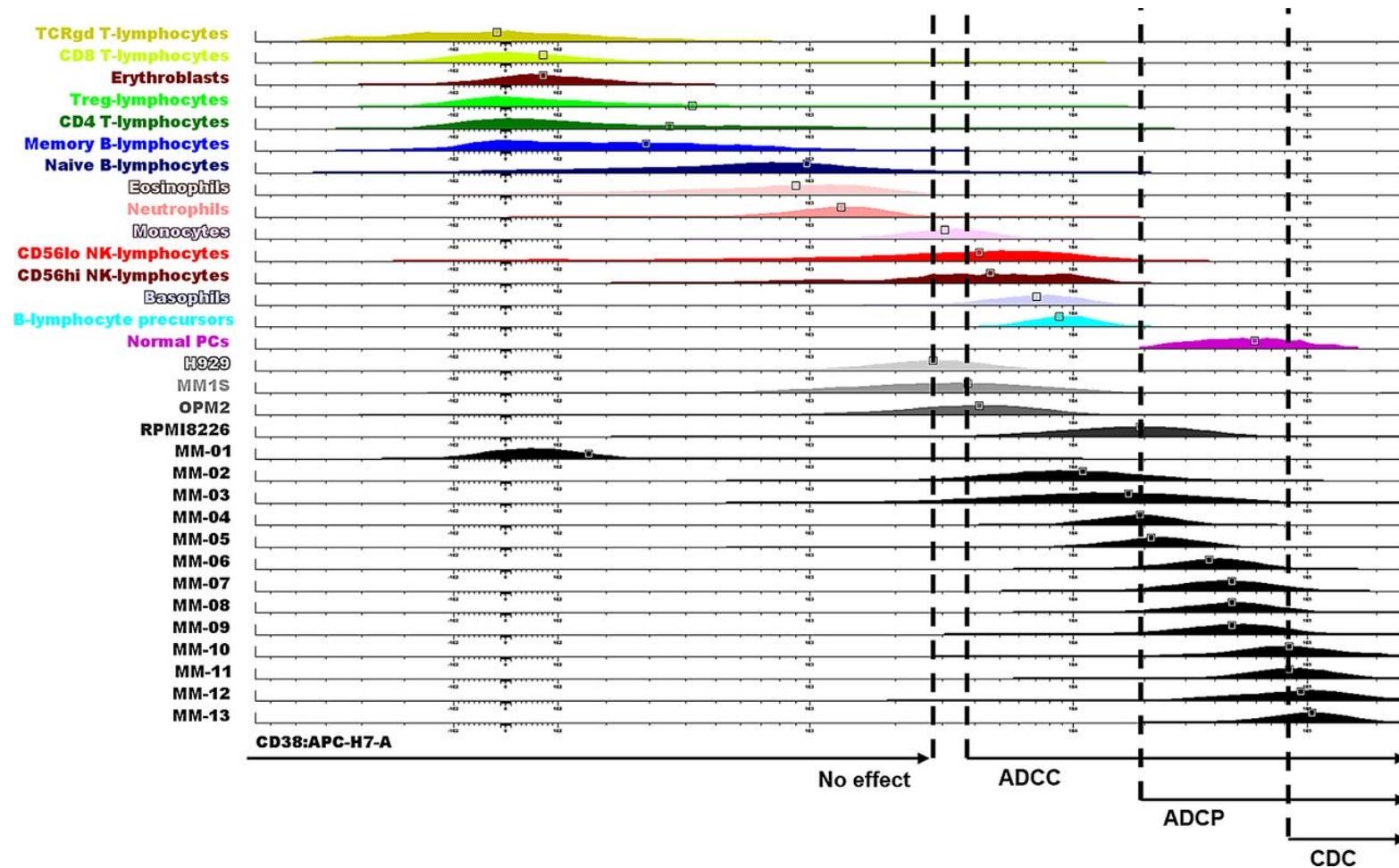


## Hierarchy of CD38 expression across immune subtypes, as assessed via flow cytometry (A) and CyTOF® (B)

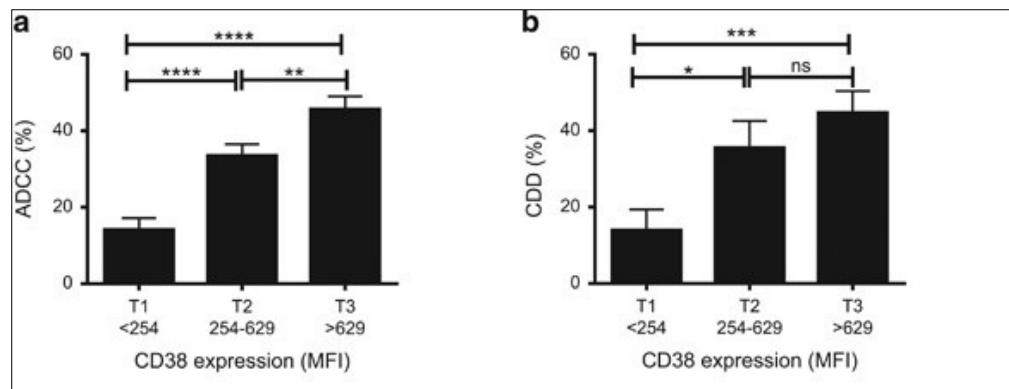
Different flow cytometry approaches on MM BM confirm comparable CD38-marker intensity in natural killer (NK), monocyte, and B- and T-cell compartments



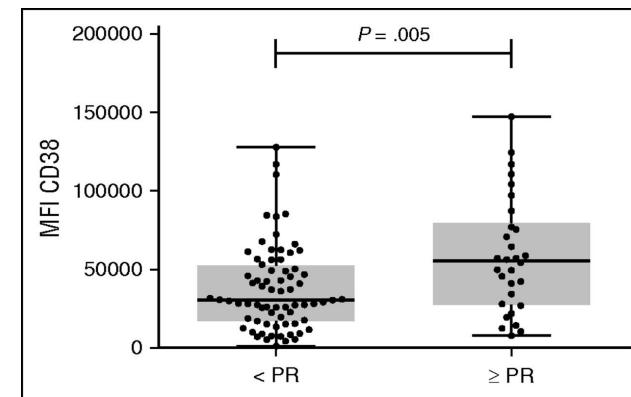
## Schematic representation of the different MOA possibly triggered by anti-CD38 antibody according to the levels of CD38 expression in normal and tumor cells



# CD38 is determinant to response to Daratumumab treatment

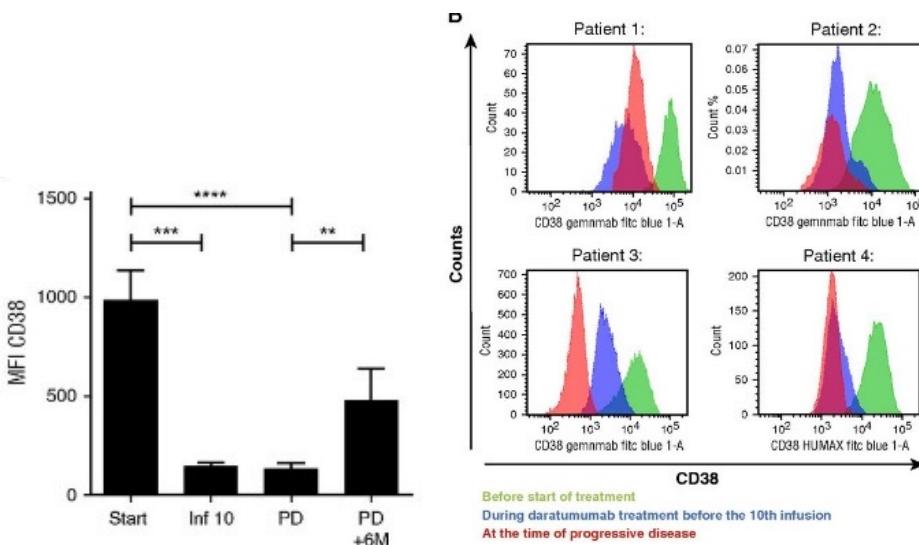


Extent of daratumumab-mediated CDC and ADCC is associated with CD38 expression levels

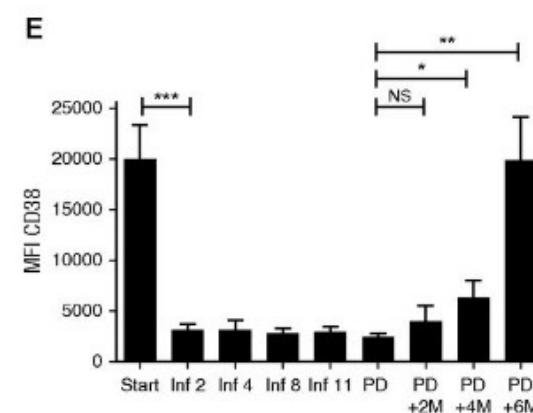


CD38 expression levels are higher in daratumumab-treated patients who achieve  $\geq$ PR

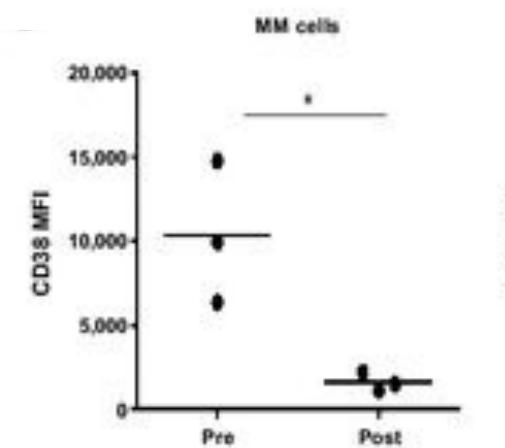
# .....but CD38 is rapidly reduced on MM cells also in patients with deep and durable responses



Bone marrow MM cells

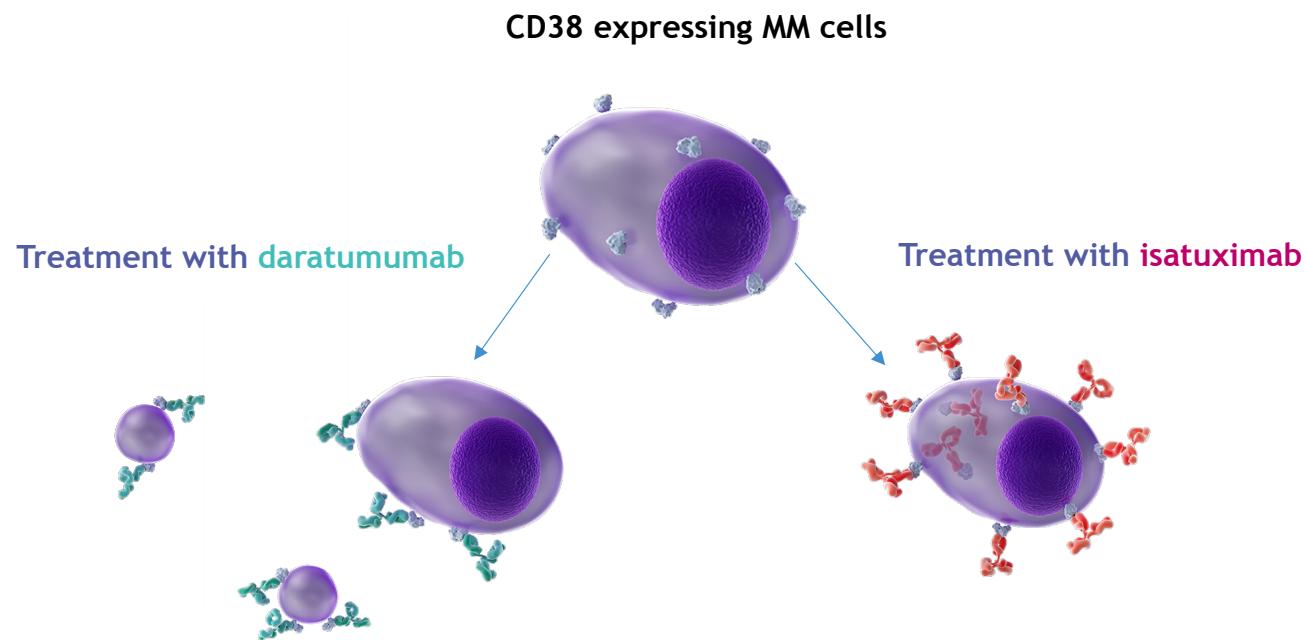


Circulating MM cells



Circulating MM cells,  
before and directly after  
first infusion

## Effect of antibody binding on CD38 expression: isatuximab does not decrease CD38 expression



- Cytotoxicity via CDC, ADCC, and ADCP is more efficient when CD38 expression is high, but the clinical relevance of reduced receptor expression is not clearly defined
- Isatuximab may induce CD38 internalization but not release of CD38 from the cell surface
- Daratumumab induces release of CD38 in microvesicles, leading to decreased CD38 expression

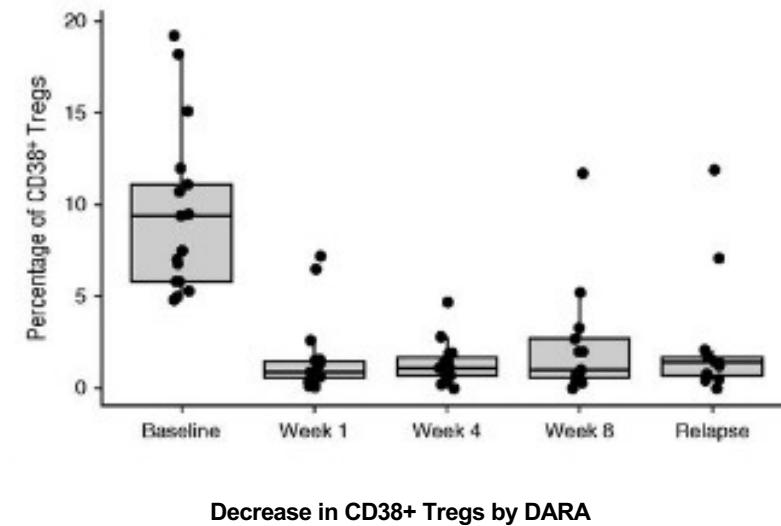
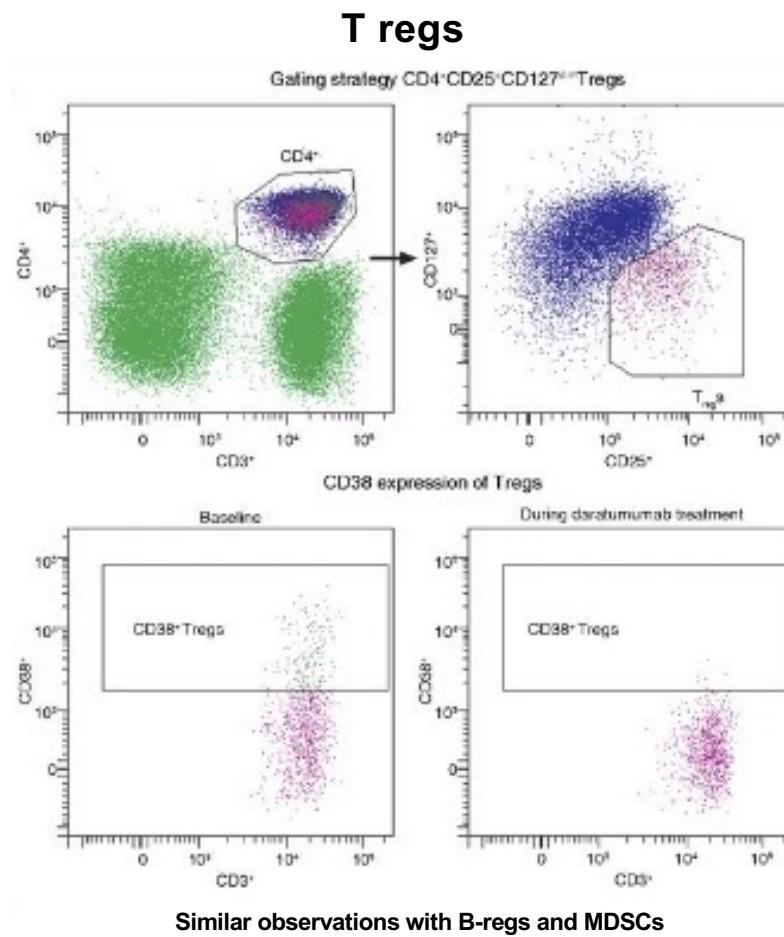
Horenstein AL et al. Cells 2015

Plesner T et al. Cells 2020

Moreno L et al. Clin Cancer Res 2019

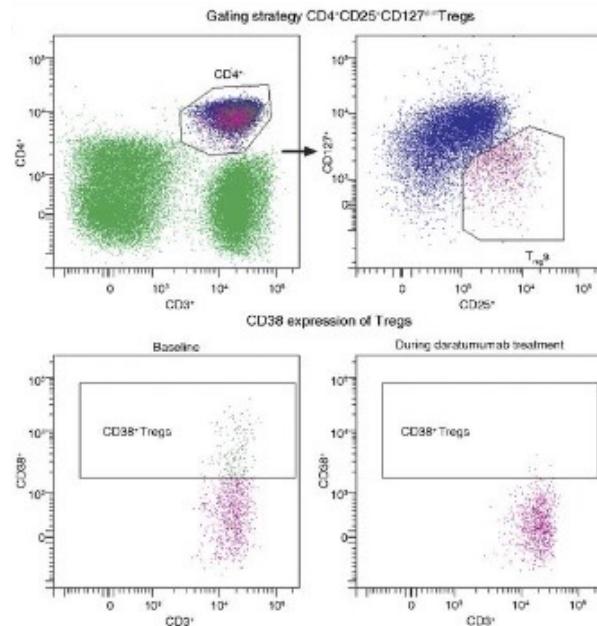
**CD38 expression on tumor cells  
does not explain the whole story**

# Daratumumab eliminates CD38-positive immune suppressor cells

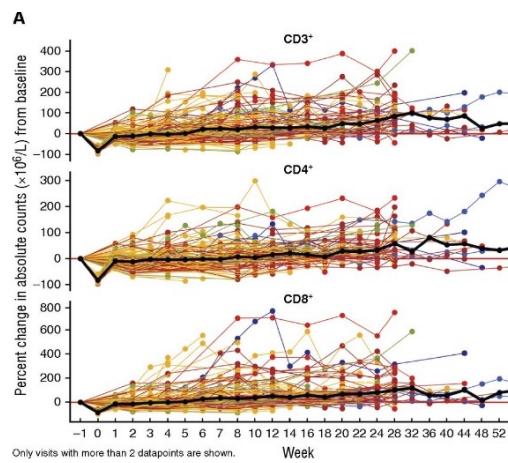


# Daratumumab has immunomodulatory effects

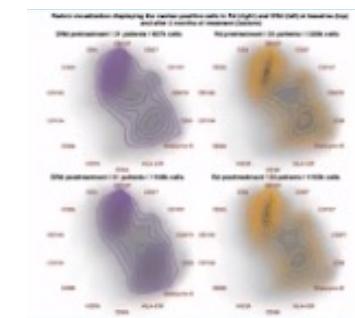
Daratumumab eliminates CD38+ Tregs, B-regcs and MDSCs



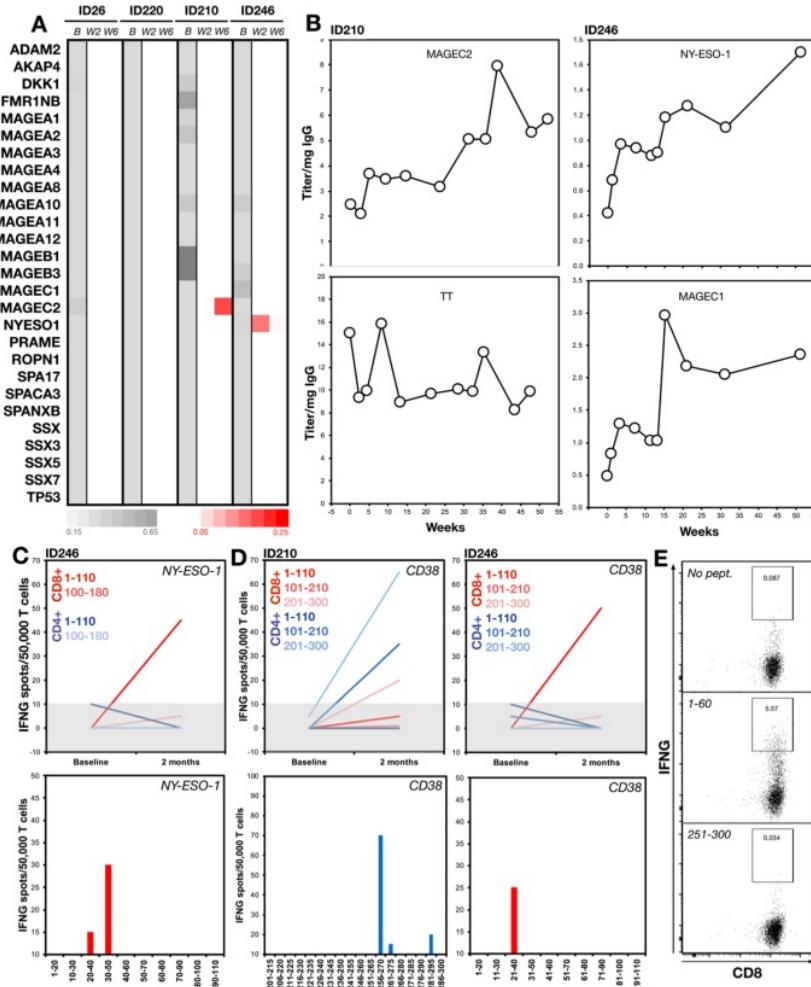
Daratumumab increases CD4+ and CD8+ T cells



Daratumumab increases killing capacity of T-cells (granzyme B)

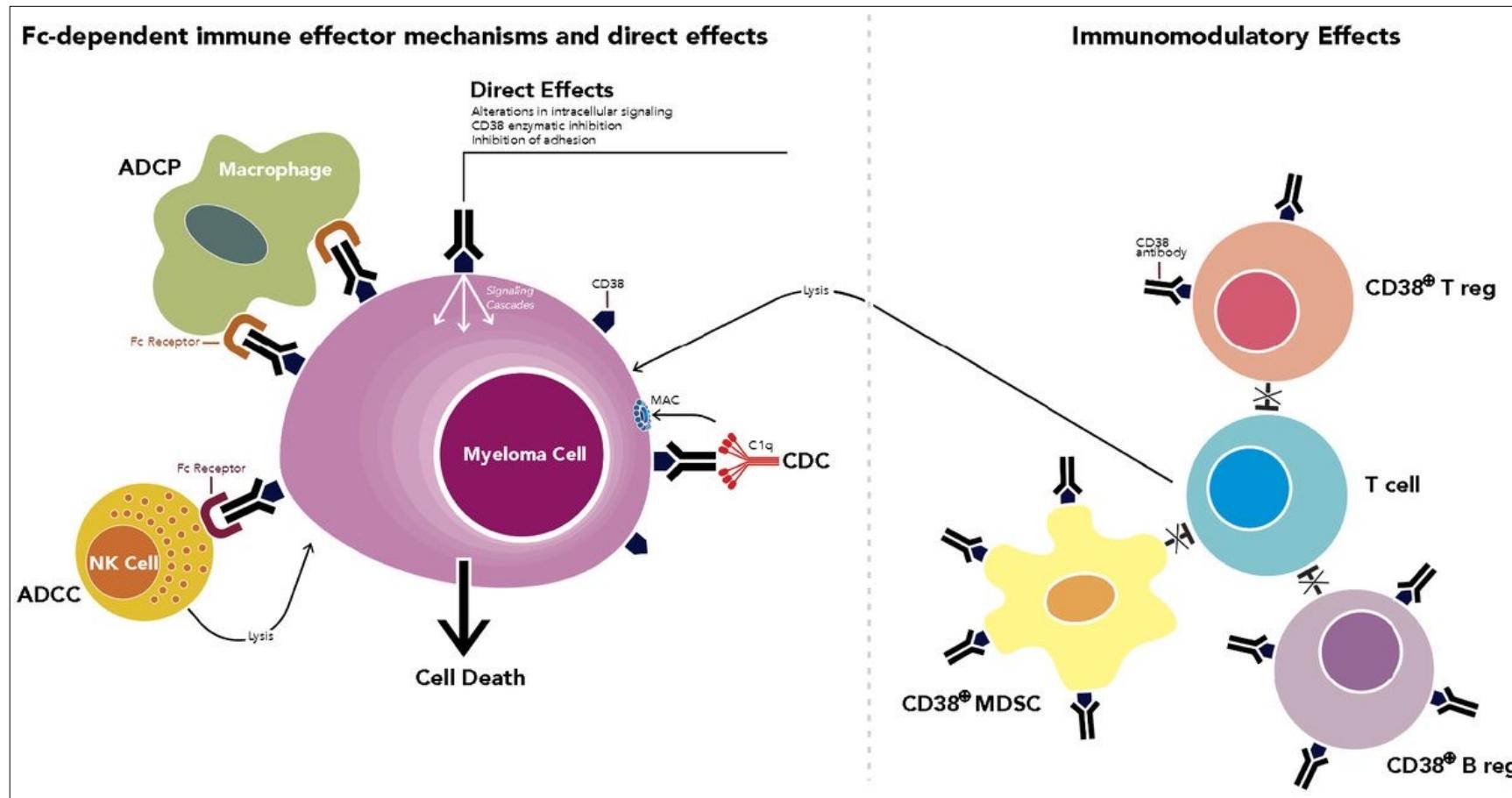


# Immunomodulation by Isatuximab



Immune responses against CD38 and other MM-associated antigens during immunotherapy with isatuximab

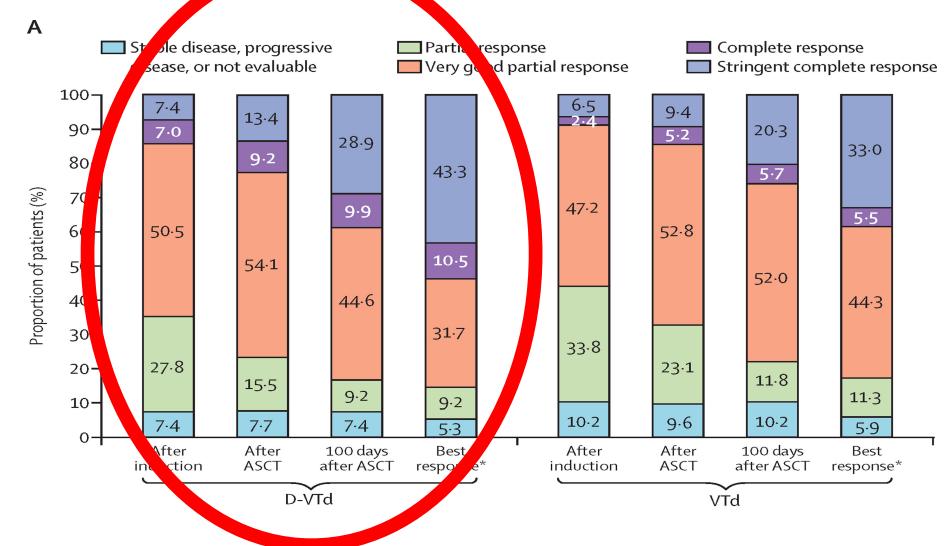
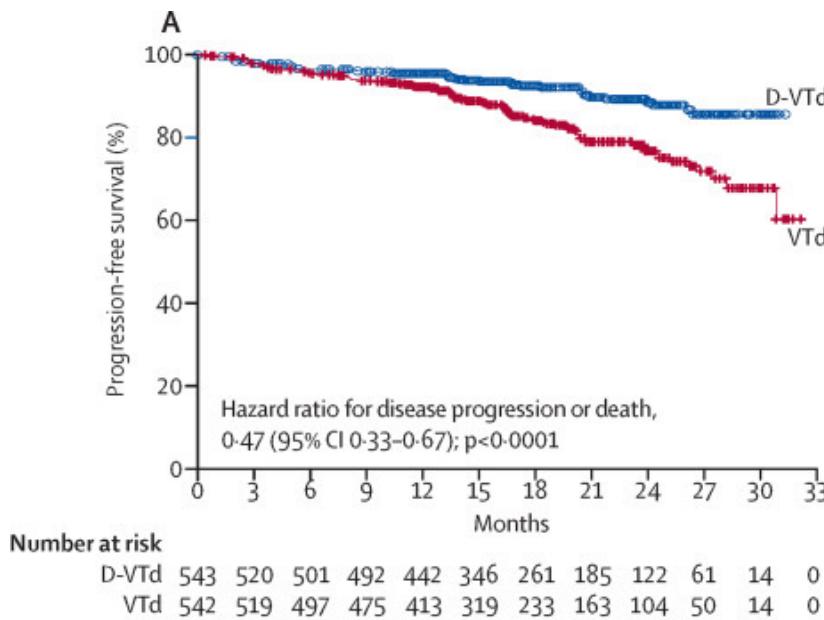
# Mechanism of action of CD38 antibodies



# **Clinical application for anti-CD38 MoAbs**

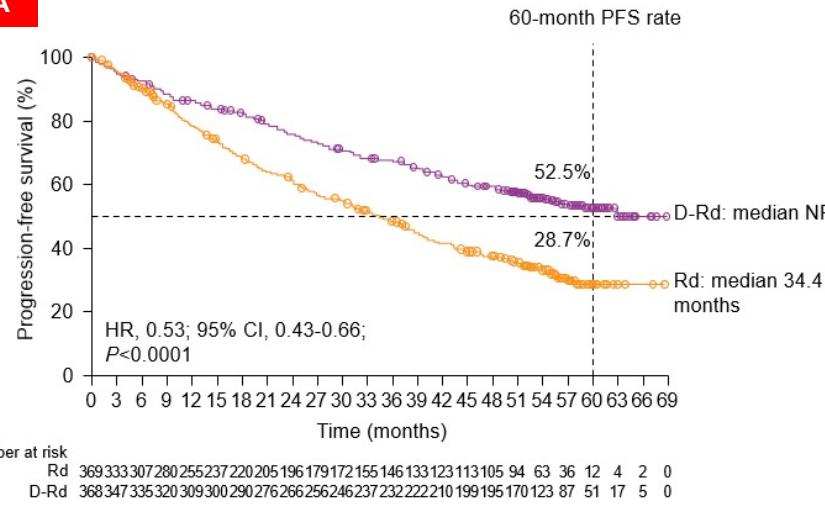
# Transplant Eligible-patients

## CASSIOPEIA TRIAL

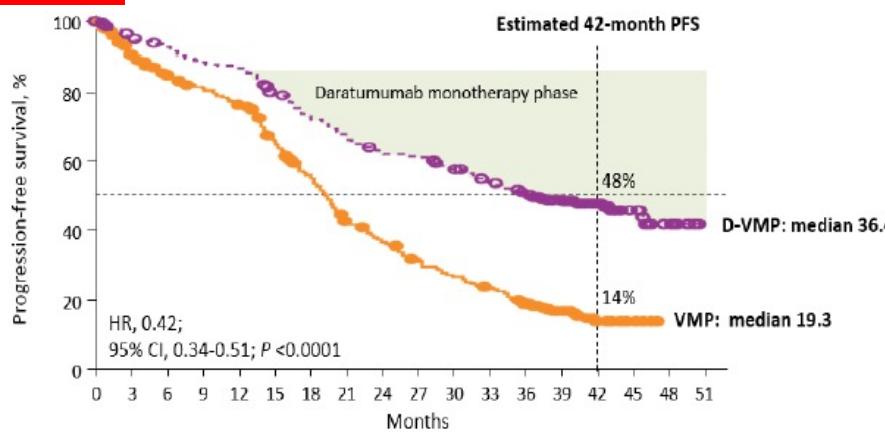


# Non transplant eligible-patients

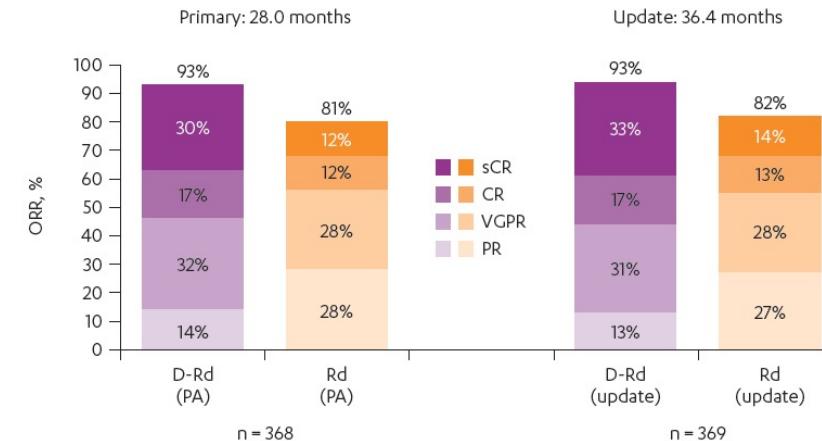
MAIA



ALCYONE

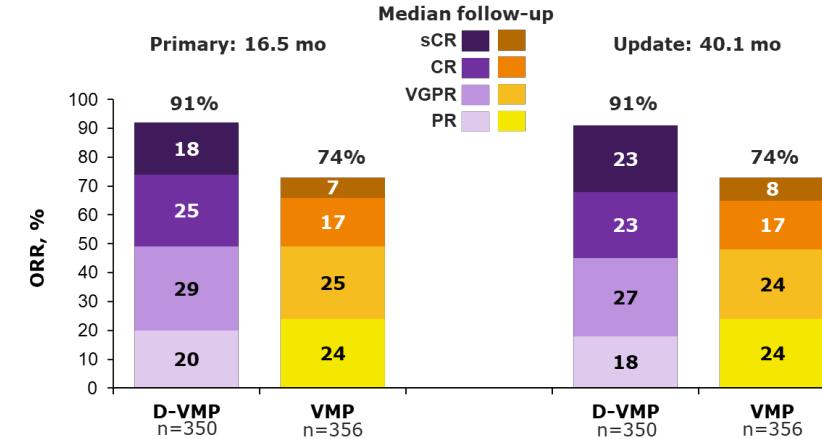


Median follow-up



Facon T et al., NEJM 2019

Median follow-up

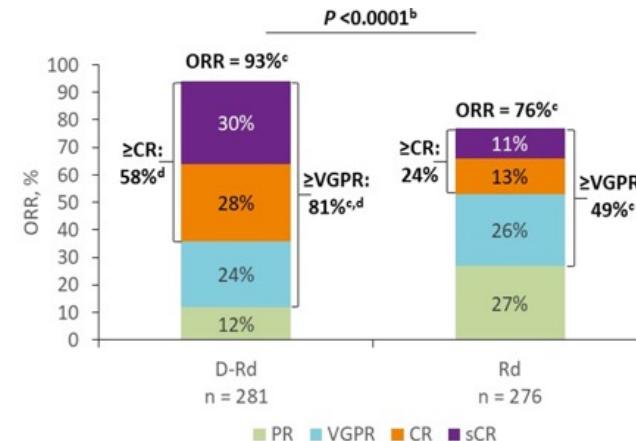
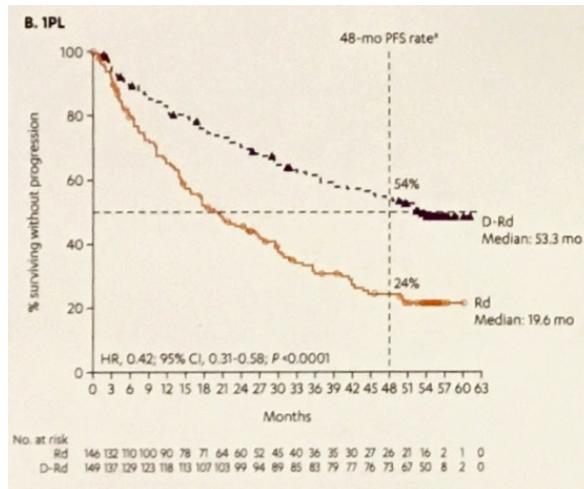


Mateos MV et al., Lancet 2020

# Clinical application for anti-CD38 MoAbs: daratumumab

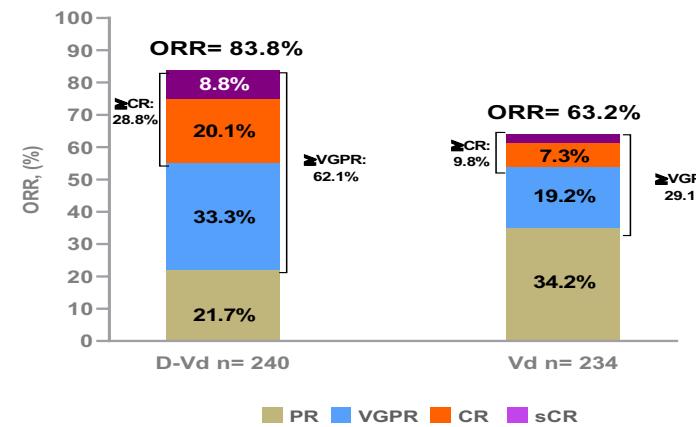
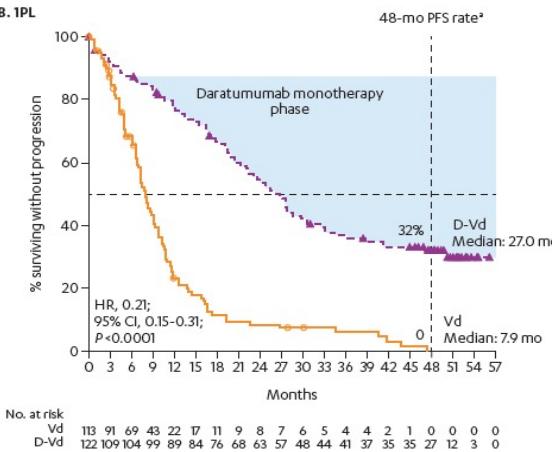
## 1<sup>st</sup> RELAPSE

**POLLUX**



Dimopoulos MA. et al NEJM 2016

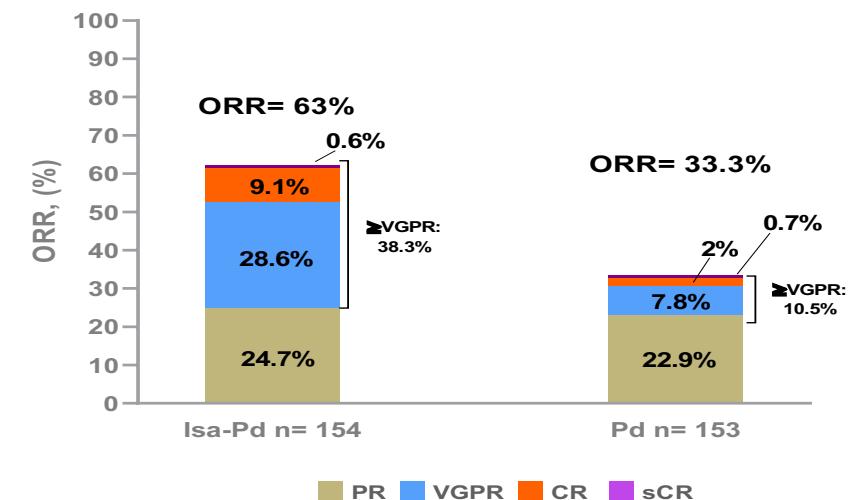
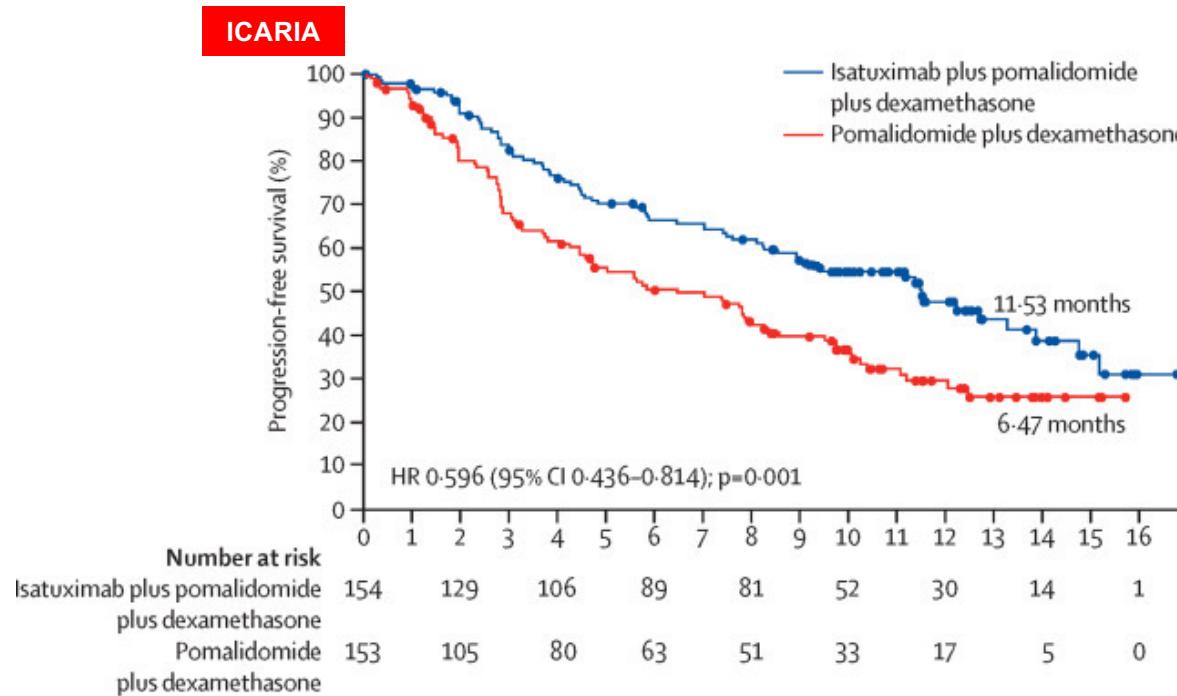
**CASTOR**



Palumbo A. et al NEJM 2016

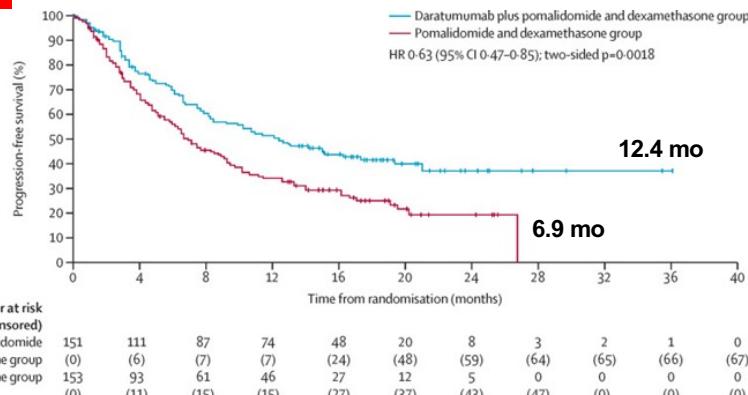
# Clinical application for anti-CD38 MoAbs: isatuximab

## 2<sup>nd</sup> RELAPSE

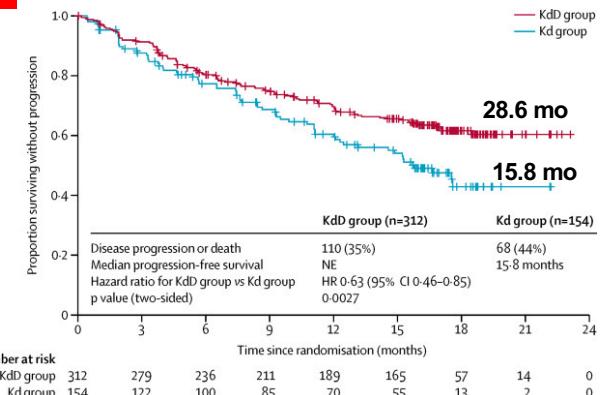


# Clinical application for anti-CD38 MoAbs: 1<sup>st</sup> relapse (phase III trials)

**APOLLO**



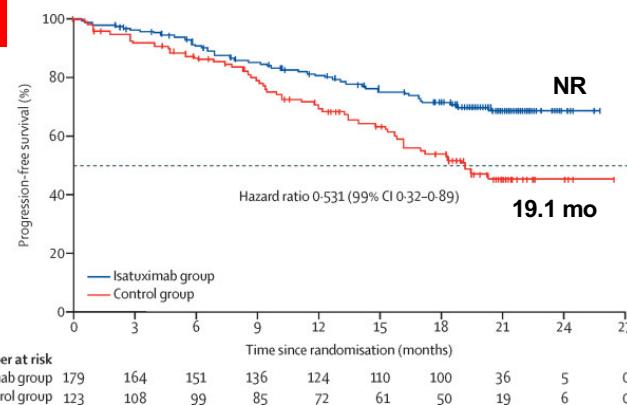
**CANDOR**



Dimopoulos MA, et al. Lancet Oncol. 2021

Dimopoulos MA, et al. Lancet. 2020

**IKEMA**



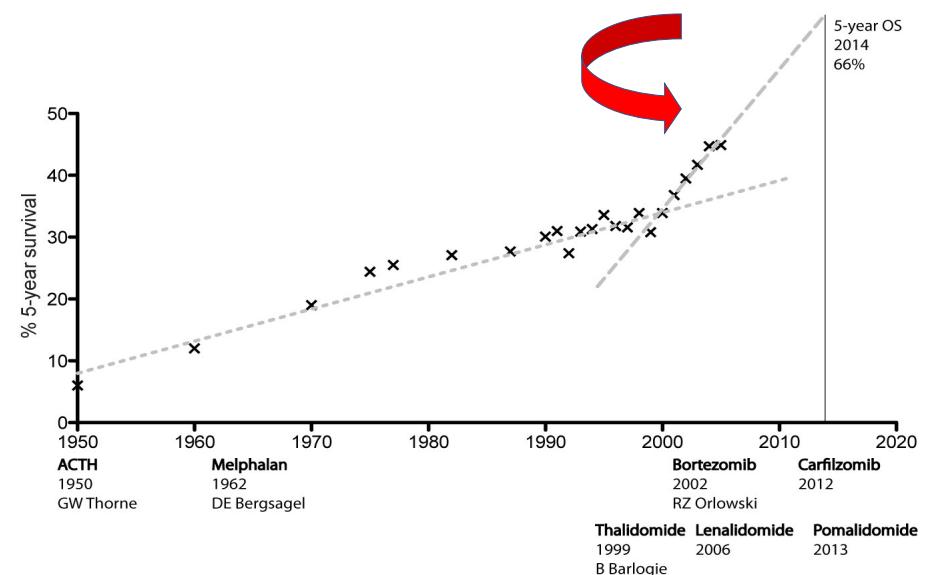
Moreau P, et al. Lancet. 2021

# CONCLUSIONS

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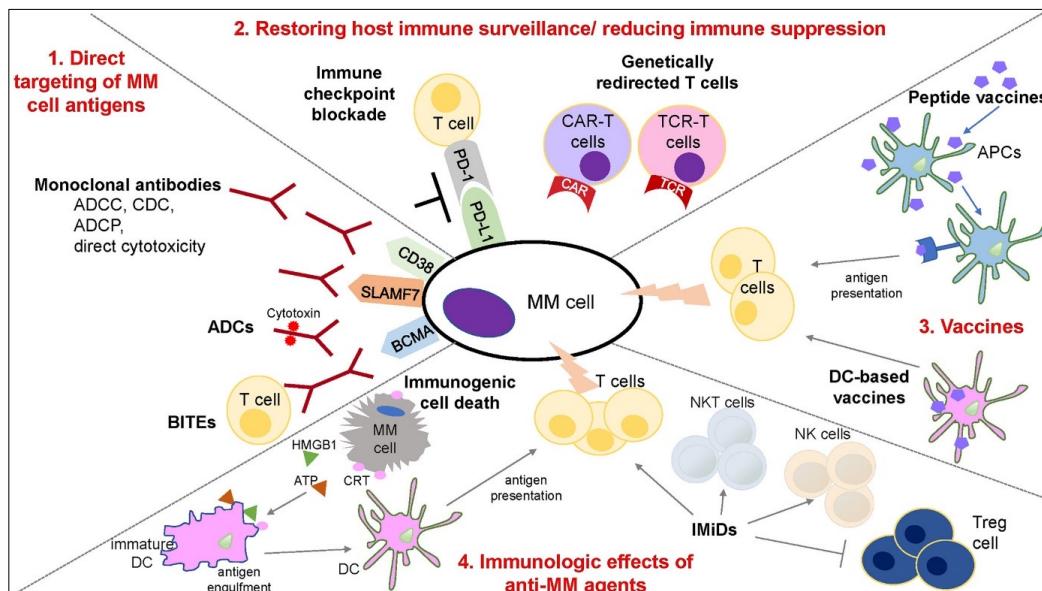
- Landscapes of immunotherapy is continuously evolving in MM thus resulting in improved clinical outcome
- The approval of MoAb against CD38 (dara/isa) in upfront and relapsed setting represents a milestone for immunotherapy of MM
- These MoAb-based agents induce cytotoxicity via multiple effector-dependent mechanisms and can further induce immunomodulation to repair a dysfunctional tumor immune microenvironment

**Immunotherapy:**  
DARA/ISA/ELO/NIVO/ADC/CAR-T/bispecific

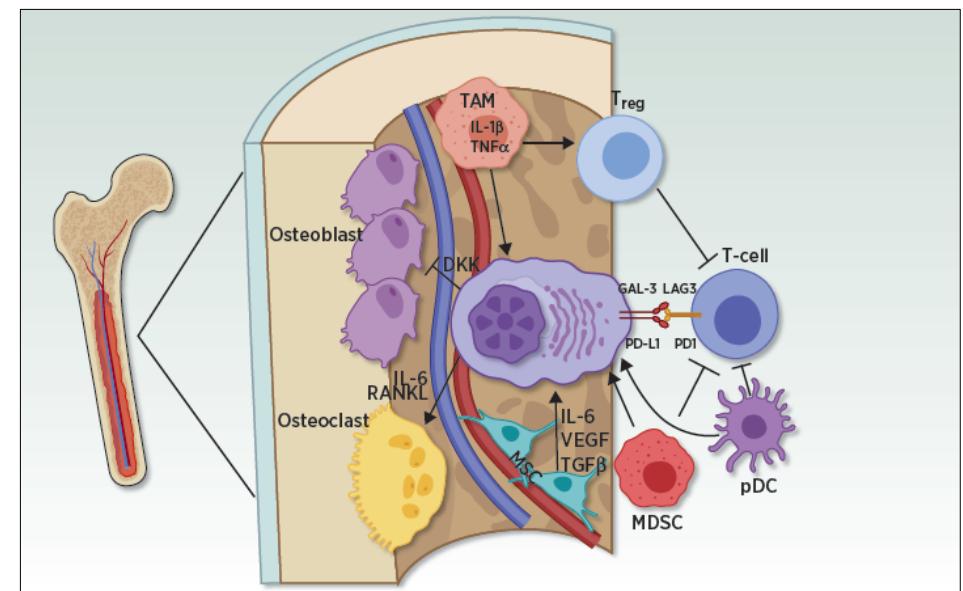




# MM cells grow and evolve within a supportive BM microenvironment



Yamamoto L. et al Front. Oncol. 2021



Sperling AS, Anderson KC Clin Cancer Res 2021